Identification and Optimization of a Liquid Medium for the Culture of *Colletotricum graminicola*

An Honors Thesis (HONRS 499)

by

Jennifer L. Minter

Thesis Advisor
Dr. James Mitchell

Ball State University
Muncie, Indiana

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Abstract

Crude carbon compounds were evaluated in several stages to determine the optimal liquid-culture medium for conidial production in *Colletotrichum graminicola.* Among those tested, use of unfiltered V8 medium resulted in optimum conidiation. % v/v of this medium was evaluated along with pH and conductivity using a central composite design to optimize conidiation in *C. graminicola.* The predicted titer using this model was $4.78x10^7$ conidia/mL, using a medium consisting of 40.14% v/v V8, with a pH of 6.16 and a conductivity of 28.03mS.

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Introduction and Rationale

A major problem for farmers has always been weeds, which has been dealt with in several ways throughout human history. In the past, farmers relied entirely on manual weeding. Many farmers, particularly in less-developed regions, rely on this technique today. However, with the recent increase in the size of farms, manual weeding quickly became impractical. The current strategy for these farmers is usually herbicides. Although they are often the only recourse, herbicides pose environmental concern. Most do not rapidly degrade in the environment and thus persist long after the weeds have been killed. They have been detected in our surface waters [1, 11], the waters that Muncie and many communities largely rely on for drinking water.

An alternative to chemical herbicides is the use of bioherbicides. Bioherbicides are organisms, usually fungi, which naturally infect the unwanted weed in the wild. Most fungi are host-specific, meaning that they only infect certain plants and leave others largely alone. A farmer can apply the organism to his weeds in order to infect and kill the plants without harming his crops. Typically, when a fungal bioherbicide is applied with the same application technologies as chemical herbicides [19], it infects and kills the weed host within 1-2 weeks. After death of the weed host, the bioherbicide organism naturally dies back to its usual numbers in the environment because its food source is depleted. In contrast to chemical herbicides, the use of fungal bioherbicides does not result in any toxic substance that could accumulate in the environment or appear as surface or groundwater contaminants.

One weed that poses major problems for farmers is johnsongrass [*Sorghum halepense* (L.) Pers.]. It is an exotic grass native to the Mediterranean region which has established itself in warm regions of all major agricultural areas of the world [13]. It has been reported as one of the world's ten worst weeds [10]. Johnsongrass reduces crop yields in corn [2, 12, 16], soybeans [23], and cotton [15]. It also hosts insect and disease pests of grain sorghum [9, 13], and interbreeds with grain sorghum [13].

Numerous chemical herbicides have been developed and tested for efficacy against johnsongrass [8, 18]. Several fungal bioherbicides have also been proposed for the control of johnsongrass [5, 6, 14, 15, 24]. One of these is *Colletotrichum graminicola* (Ces.) Wils., which causes anthracnose [14], a plant disease. This organism has also been proposed as a means to control barnyard grass [*Echinochloa crus-galli*], a common weed problem in rice [26].

Only three fungal bioherbicides have been registered for use in North America [17, 20]. One of the three fungal bioherbicides was Collego, which contained a different species of *Colletotrichum*; *C. gloeosporioides* [3, 20]. These fungi infect by using spores, a dormant product of fungal reproduction, which germinate on the plant and infect it. Low-cost methods for producing infective spores must be used in order for a profit to be maintained. Submerged-culture fermentations are currently considered to be the most economical method of production [7]. In this method, the fungus grows and produces spores in a liquid medium. This study aimed to develop an optimized submerged-culture medium for the sporulation of *Colletotricum graminicola.*

Over the course of four months, many media were tested. To begin, basic liquids were tried, such as the brine juice in canned vegetables, V8, sugar solutions, coffee, and

tea. Based on the results of that experiment, V8 juice, corn syrup, and canned pea brine were selected for further testing. Subsequent experiments tested various concentrations of those media and also tested several additives to the basic media, including the ingredients in Collego medium that were effective for the other species of *Colletotrichum*. The results eliminated corn syrup and pea brine from the possibilities. After determining that unfiltered V8 led to more spores than filtered V8, this medium was used in the final experiment. The final experiment made use of a statistical tool in the JMP4 software which predicted the optimum conditions for the best sporulation of the fungus. The conditions tested were V8 concentration, pH, and conductivity. Optimal sporulation was predicted at 40.14% V8 by volume with a pH of 6.16 and a conductivity of 28.03mS. The predicted amount of spores with these conditions was 47.8 million spores per milliliter. The results indicate a very promising spore production in this medium, but more research would be necessary to determine the effect the spores produced in this medium would have on the johnsongrass plant.

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Jennifer L. Minter
Honors Thesis Credit (HONRS 499)
Department of Biology, Ball State University, Muncie, IN 47306, USA
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Introduction

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Numerous herbicides have been developed and tested for efficacy against johnsongrass [8, 18]. Although they are often the only recourse for farmers, herbicides pose environmental concern because many do not rapidly degrade and have been detected in surface waters [1, 11].

An alternative to chemical herbicides is the use of bioherbicides. Typically, when a fungal bioherbicide is applied as inundative inoculum with the same application technologies as chemical herbicides [19], it infects and kills the weed host within 1-2 weeks. After death of the weed host, the bioherbicide organism is naturally reduced in numbers to background levels. In contrast to chemical herbicides, the use of fungal bioherbicides does not result in any toxic substance that could accumulate in the environment or appear as surface or groundwater contaminants.

Several fungal bioherbicides have been proposed for the control of johnsongrass [5, 6, 14, 15, 24]. One of these is *Colletotrichum graminicola* (Ces.) Wils., which causes anthracnose [14]. This organism has also been proposed as a means to control barnyard grass [*Echinochloa crus-galli*], a common weed problem in rice [26].

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Materials and Methods

Organism

Single-spore isolates of *C. graminicola* were collected at locations in Arkansas and Texas. Stock cultures were maintained on both potato-dextrose agar slants under mineral oil and glycerol-skim milk at -80°C. The inoculum conidia were produced on Torula yeast agar (TA). The TA medium contained: 15g. Torutein-10 (Provesta, Hutchinson, MN), 15g M-100 (Grain Processing Corporation, Muscatine, IA), 1.0g. K_2HPO_4, 0.5g. $MgSO_4$ x $7H_2O$, and 15g. agar (Difco, Detroit, MI) per liter of deionized water. For each experiment, 5 plates of TA medium were inoculated and incubated on a laboratory bench for 7 days at 22-24°C under fluorescent lights (1:1, Gro-Lux: Cool White) adjusted to a 14-hour photoperiod. The TA medium plates were

aseptically scraped with sterile cotton swabs and conidia were suspended in 10mL sterile deionized water.

Submerged culture

Liquid culture experiments were conducted using 250-mL Erlenmeyer flasks, each containing 50mL medium. An appropriate volume of inoculum was introduced to the autoclaved (15-minute liquid cycle) media, resulting in an initial spore concentration of $2x10^4$ conidia/mL. Cultures were incubated at 22-24°C on a rotary shaker at 220rpm. Flasks were manually shaken daily to remove aerial mycelial growth on the flask wall. Conidia were counted with a hemacytometer under the microscope after 6 days of culture unless indicated otherwise.

Media and experimental design

Crude carbon sources shown in Table 1 were evaluated for sporulation of the fungus. Canned vegetables, including V8 juice unless indicated otherwise, were filtered through four layers of grade 40 cheesecloth and the filtrate (brine) was used in the experiment. Coffee was prepared on a Mr. Coffee automatic-drip coffeemaker using the amount recommended by the manufacturer. Tea was prepared by placing 3 tea bags in 1L boiling water for 20min. Dilutions of concentrated media were made using deionized water. Later experiments tested TA liquid medium, which was prepared similarly to the solid medium with the omission of the agar. COLLEGO medium was also evaluated, which was prepared from the following ingredients: 5g. KNO_3, 2.5g. K_2HPO_4, 1.25g $MgSO_4 \times 7H_2O$, 10g sucrose (Difco, Detroit, MI), 0.01g $FeCl_2$, 75mL V8 juice, and 425mL deionized water. This basic formulation contains 15% V8 v/v; alterations of the formula were tested using 30% V8 v/v and 15% Pea v/v. Thirty percent V8 and 15% Pea were also tested using 3g/L. $CaCO_3$ as an additive. When adjustments of pH were required, 50% NaOH and 1:10 and 1:100 dilutions were used to increase pH and concentrated HCl and 1:10 dilution were used to decrease pH.

Results

Nineteen carbon sources were evaluated for conidia production and shape (Table 1). Sporulation was observed in all concentrations of corn syrup, V8, and Pea. Greatest numbers were observed in V8 and Pea, but both media produced oval conidia. The corn syrup produced falcate conidia, similar to the inoculum conidia, so it was also selected for further study. Raw data from this experiment are shown in appendix A.

TA medium in liquid form was evaluated (100 and 33% v/v) along with dilutions of Pea and filtered V8 (5, 10, 15, 20, and 30% v/v). This experiment was conducted in quadruplicate. Greatest numbers were again observed in V8 and Pea; TA and corn syrup were dropped from further consideration. Raw data and analysis of this experiment are shown in appendix B.

The additive ingredients of Collego, a medium developed for a different species of *Colletotrichum*, were tested with this organism using selected carbon sources, and $CaCO_3$ was tested for it's efficacy as an additive. The experiment was performed with 8 replicates per treatment. It was noted during counting that some of the treatments exhibited evidence of conidial germination. There was no significant difference between 30% filtered V8 and 30% filtered V8 with Collego additives (Table 2). These two treatments were significantly different from all Pea treatments and all treatments with the $CaCO_3$ additive ($p<0.05$) and were selected for further testing. Raw data and analysis of this experiment are shown in appendix C.

Thus far, all experiments had been conducted using filtered V8. Comparisons were made between the effects of filtered versus unfiltered V8 in both plain media and media augmented with Collego additives at 15 and 30% v/v concentrations. This experiment was performed with 8 replicates per treatment. Because of the earlier observation of conidial germination on day 6 of culture, daily counts were also performed to determine the optimal harvesting period for this organism. Conidial counts leveled out around day 5 for all

Table 1: Crude carbon sources evaluated for sporulation of *Colletotrichum graminicola*

Type (concentration)	Specific ingredient
Canned vegetable brine (5, 10, and 15% v/v)	V8 juice [a], sliced carrots[b], butter beans[b], mustard greens[b], golden hominy[b], whole kernel golden corn (no salt)[b], sliced Irish potatoes[b], cut yams[b], leaf spinich[b], pinto beans[b], green shelled blackeye peas[b], cut beets[b], peas[f], green beans[b] and cut okra[c]
Syrups (0.2, 0.5, and 1% w/v)	Corn (Karo dark)[g], Molasses[h]
Coffee and Tea (10, 30, and 100% v/v)	Guatemalan blend coffee[d], regular tea[c]

[a]Campbell Soup Co. (Cambden, NJ)
[b]Marsh Supermarkets, LLC (Indianapolis, IN)
[c] Bruce Foods Corporation (New Iberia, LA)
[d]Alliance World Coffees (Muncie, IN)
[e]Lipton (Englewood Cliffs, NJ)
[f]DelMonte Foods (San Francisco, CA)
[g]EPC Int. (Englewood Cliffs, NJ)
[h]B&G Foods (Roseland, NJ)

treatments (Figure 1), so flasks were counted at day 5 instead of day 6 from this point forward. Conidia counts were significantly higher for three of the unfiltered treatments (V8 30 and 15% v/v and 30% v/v V8 with Collego additives) than for the fourth unfiltered treatment and all filtered treatments (Table 3). Unfiltered plain V8 was selected for use in the final optimization experiment. Raw data and analysis of this experiment are shown in appendix D.

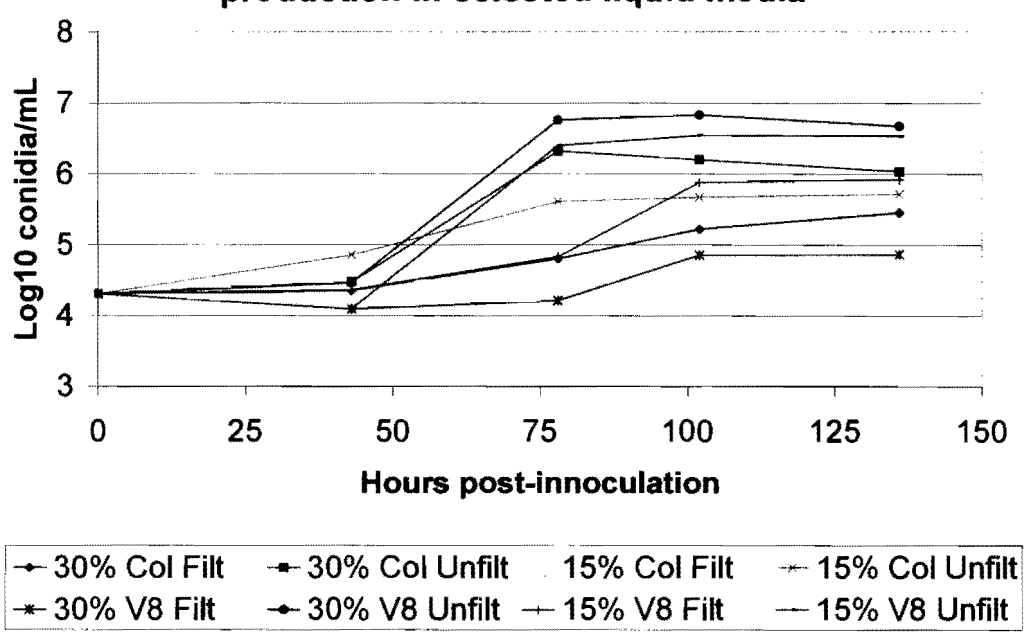
Table 2: *Colletotrichum graminicola* conidia production in crude liquid media with selected additives

Medium	Mean log_{10} conidia/mL	
30% v/v filtered V8 with 3g/L $CaCO_3$	3.38	c
COLLEGO	3.52	bc
15% v/v Pea with Collego additives	3.62	bc
15% v/v Pea with 3g/L $CaCO_3$	3.72	bc
15% v/v Pea	4.11	b
30% v/v filtered V8 with Collego additives	5.27	a
30% v/v filtered V8	5.82	a

Table 3: *Colletotrichum graminicola* conidia production in crude filtered or unfiltered media with or without Collego additives

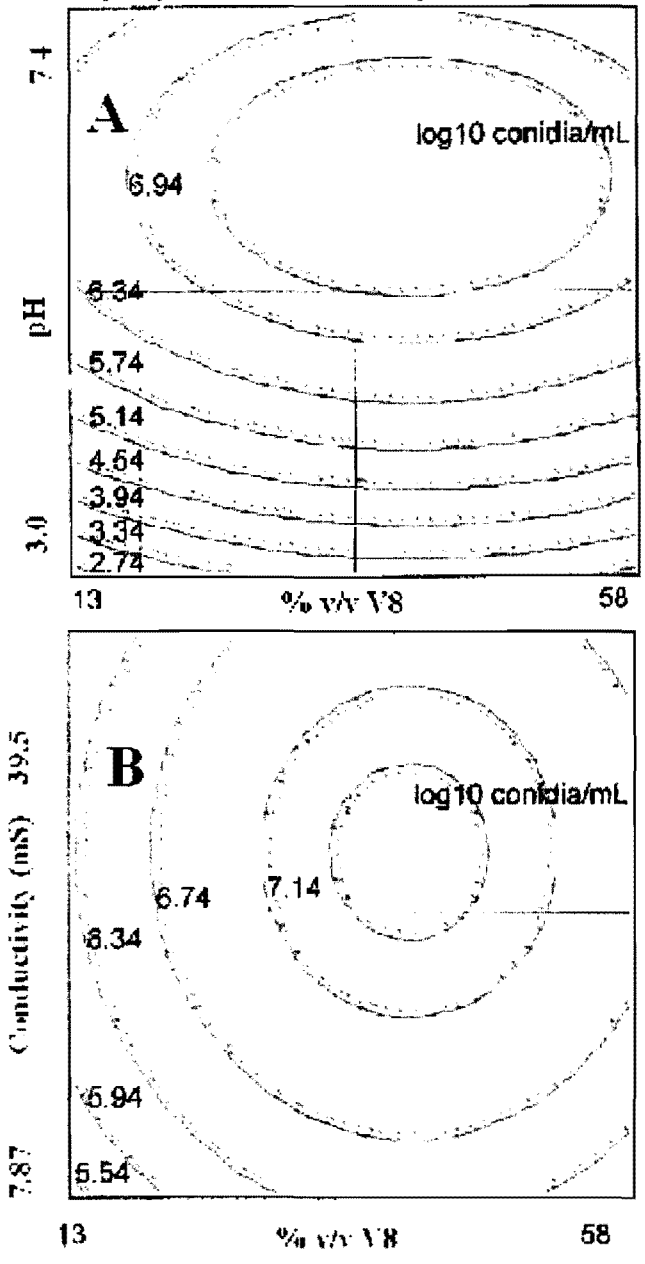
Medium and Concentration	Mean Log_{10} conidia/mL	
15% v/v filtered V8 with Collego additives	3.79	d
15% v/v unfiltered V8 with Collego additives	5.51	bcd
30% v/v filtered V8 with Collego additives	4.76	cd
30% v/v unfiltered V8 with Collego additives	5.94	abc
15% v/v filtered V8	5.15	bcd
15% v/v unfiltered V8	6.16	ab
30% v/v filtered V8	4.66	bcd
30% v/v unfiltered V8	6.69	a

Figure 1: *Colletotrichum graminicola* conidia production in selected liquid media



An orthogonal CCD in the JMP4 software was utilized to optimize conidia production with the following variables: unfiltered V8 concentrations, pH, and conductivity. Prior to conducting the experiment, pH and conductivity were standardized over a wide range of V8 concentrations pre- and post- autoclave in order to predict starting pH and conductivity from desired post-autoclave pH and conductivity.

Figure 2: Contour plots of pH and conductivity interaction with % v/v V8 to affect spore yield of *Colletotrichum graminicola*



Conductivity adjustments were made with KCl ranging from 0% w/v to 0.02% w/v. Results of this standardization are shown in appendix E. The r^2 value was 0.97. Conidial concentration results for V8 versus pH a V8 versus conductivity are shown in Figure 2. Optimal sporulation was predicted at 40.14% v/v V8 with a pH of 6.16 and a conductivity of 28.03mS. The predicted spore titer with these conditions was 4.78×10^7 conidia/mL. Raw data and analysis of this experiment are shown in appendix F.

Discussion

Fungi differ by species in optimal medium for sporulation. As seen in this study, the Collego medium, optimal for *Colletotrichum gloeosporioidies*, is not the optimal medium for *C. graminicola*. The orthogonal CCD predicted a spore titer of 4.78×10^7 conidia/mL with 40.14% v/v V8, a pH of 6.16 and a conductivity of 28.03mS. This titer would be reached after 5 days of incubation at 22-24°C. It is possible that other factors not considered in this study could affect conidia production, and if considered, lead to a greater spore titer. However, the titer predicted by the orthogonal CCD model is satisfactory and further extensive testing would only be necessary if it were determined that a higher initial titer would be required for economical production.

It must be remembered that the overall goal of this study is to develop a liquid-culture medium that has the potential to be used in the future to produce conidia for use as a bioherbicide against johnsongrass. As noted earlier, conidia shape in V8 is oval, not falcate as is produced on solid media. This has been observed by others [4, 22] and the effects on spore germination have been studied [4]. It was found that oval conidia germinated similarly to falcate conidia and had the additional advantage of having less strict requirements of surface hydrophobicity. Further tests would need to be done to confirm that finding with this medium and to determine the virulence of the conidia produced by this medium on the johnsongrass plant.

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Appendix A: Carbon Source Screening

Table 4: Conidiation in crude media and observations

Flask Number	Flask Contents	Conidia Present	Observations
1	Beets 5%	-	
2	Beets 10%	-	
3	Beets 15%	-	
4	Blackeye Peas 5%	-	
5	Blackeye Peas 10%	-	
6	Blackeye Peas 15%	-	
7	Hominy 5%	-	
8	Hominy 10%	-	
9	Hominy 15%	-	
10	Potatoes 5%	-	
11	Potatoes 10%	+	Few, all oval
12	Potatoes 15%	-	
13	Carrots 5%	+	Very few, mix of oval and falcate
14	Carrots 10%	-	
15	Carrots 15%	-	
16	Spinach 5%	-	
17	Spinach 10%	-	
18	Spinach 15%	-	
19	Peas 5%	+	Mix of oval and falcate, few
20	Peas 10%	+	Many, most oval
21	Peas 15%	+	Many, most oval
22	Butter Beans 5%	+	Very few, all falcate
23	Butter Beans 10%	-	
24	Butter Beans 15%	-	
25	V8 5%	+	Many, most falcate, some oval
26	V8 10%	+	Many, mix of oval and falcate
27	V8 15%	++	Very many, mix of oval and falcate
28	Mustard Greens 5%	-	
29	Mustard Greens 10%	-	
30	Mustard Greens 15%	+	oval conidia
31	Okra 5%	+	Many, all oval
32	Okra 10%	-	
33	Okra 15%	+	Many, all oval
34	Pinto Beans 5%	-	
35	Pinto Beans 10%	+	Very few
36	Pinto Beans 15%	-	
37	Corn 5%	-	

Flask Number	Flask Contents	Conidia Present	Observations
38	Corn 10%	+	oval conidia
39	Corn 15%	+	Many, all oval
40	Green Beans 5%	-	
41	Green Beans 10%	-	
42	Green Beans 15%	-	
43	Yams 5%	-	
44	Yams 10%	-	
45	Yams 15%	+	oval and falcate conidia
46	Corn Syrup 0.2%	+	Little vegetative biomass
47	Corn Syrup 0.5%	+	All falcate
48	Corn Syrup 1%	+	Little vegetative biomass
49	Molasses 0.2%	+	Many, all oval
50	Molasses 0.5%	+	
51	Molasses 1%	-	
52	Coffee 10%	-	
53	Coffee 30%	-	
54	Coffee 100%	-	
55	Tea 10%	-	
56	Tea 30%	-	
57	Tea 100%	-	

Table 5: Conidia counts in selected crude media

Flask Number	Flask Contents	Log_{10} Conidia/mL
46	Corn Syrup 0.2%	4.19
47	Corn Syrup 0.5%	4.03
48	Corn Syrup 1%	4.43
49	Molasses 0.2%	4.29
50	Molasses 0.5%	4.75
25	V8 5%	4.49
26	V8 10%	4.89
27	V8 15%	5.36
31	Okra 5%	4.21
32	Okra 10%	3.22
33	Okra 15%	4.55
19	Peas 5%	4.88
20	Peas 10%	5.05
21	Peas 15%	5.30

Appendix B: V8, Corn Syrup, Pea, and TA

Table 6: Explanation of Abbreviations

Medium	Corn Syrup (% w/v)					Pea (% v/v)					V8 (% v/v)					TA (% v/	
	0.2	0.5	1.0	1.5	2.0	5	10	15	20	30	5	10	15	20	30	100	3
Abbrev.	C1	C2	C3	C4	C5	P1	P2	P3	P4	P5	V1	V2	V3	V4	V5	T1	T

Table 7: Raw data of conidia counts in selected concentrations of V8, Corn Syrup, Pea, and TA

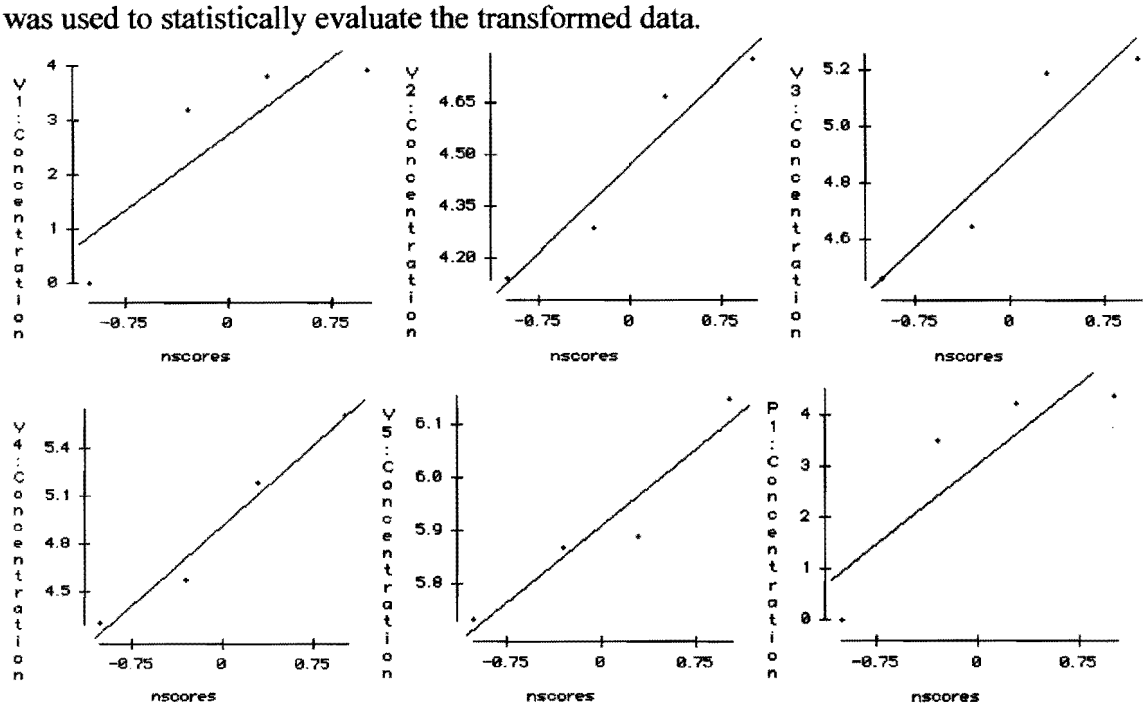
Flask Number	Flask Contents	Log_{10} conidia/mL
1	P1	0
2	P1	4.38
3	P1	4.22
4	P1	3.52
5	P2	4.81
6	P2	4.53
7	P2	4.52
8	P2	4.24
9	P3	0
10	P3	4.84
11	P3	4.94
12	P3	5.76
13	P4	5.23
14	P4	5.83
15	P4	5.19
16	P4	4.81
17	P5	5.82
18	P5	5.67
19	P5	5.1
20	P5	5.63
21	V1	3.82
22	V1	0
23	V1	3.22
24	V1	3.92
25	V2	4.29
26	V2	4.67
27	V2	4.78
28	V2	4.14
29	V3	5.19
30	V3	5.24
31	V3	4.65
32	V3	4.46

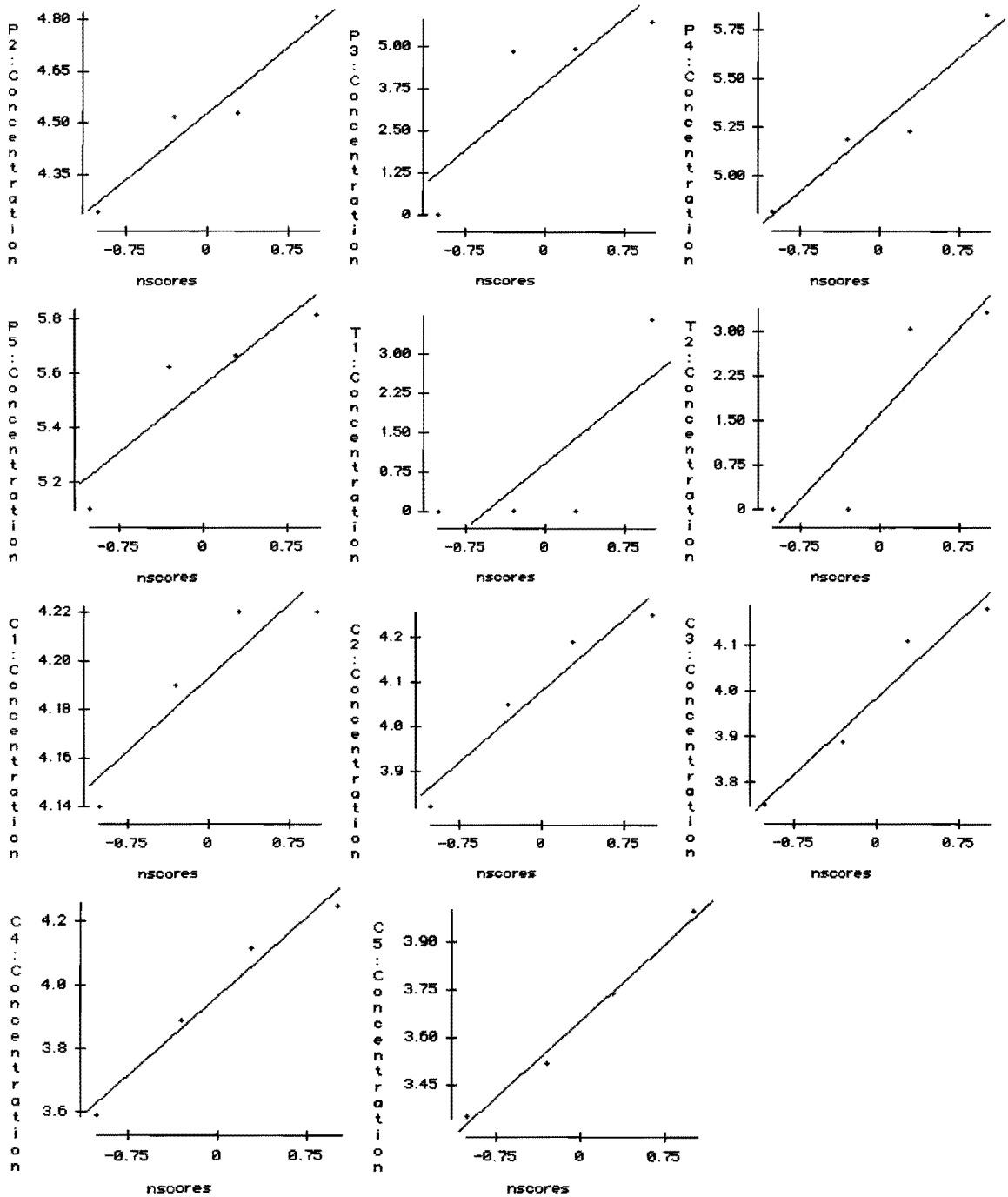
Flask Number	Flask Contents	Log_{10} conidia/mL
33	V4	4.58
34	V4	5.61
35	V4	5.19
36	V4	4.3
37	V5	5.73
38	V5	5.87
39	V5	6.15
40	V5	5.89
41	C1	4.22
42	C1	4.14
43	C1	4.22
44	C1	4.19
45	C2	4.19
46	C2	4.05
47	C2	3.82
48	C2	4.25
49	C3	4.18
50	C3	4.11
51	C3	3.75
52	C3	3.89
53	C4	4.25
54	C4	3.59
55	C4	4.12
56	C4	3.89
57	C5	4
58	C5	3.52
59	C5	3.35
60	C5	3.74
61	T1	0
62	T1	0
63	T1	0
64	T1	3.65
65	T2	0
66	T2	3.35
67	T2	3.05
68	T2	0

Summary of **Concentration**
For categories in **Medium**
No Selector

Group	Count	Mean	Median	StdDev	Min	Max	Skewness
C1	4	4.1925	4.205	0.0377492	4.14	4.22	-0.737887
C2	4	4.0775	4.12	0.191028	3.82	4.25	-0.581849
C3	4	3.9825	4	0.198221	3.75	4.18	-0.177076
C4	4	3.9625	4.005	0.289525	3.59	4.25	-0.386925
C5	4	3.6525	3.63	0.281351	3.35	4	0.218205
P1	4	3.03	3.87	2.05423	0	4.38	-1.04588
P2	4	4.525	4.525	0.232737	4.24	4.81	-6.66134e-15
P3	4	3.885	4.89	2.62259	0	5.76	-1.06691
P4	4	5.265	5.21	0.421545	4.81	5.83	0.440355
P5	4	5.555	5.65	0.314166	5.1	5.82	-0.914134
T1	4	0.9125	0	1.825	0	3.65	1.1547
T2	4	1.6	1.525	1.85158	0	3.35	0.0130972
V1	4	2.74	3.52	1.85264	0	3.92	-1.06277
V2	4	4.47	4.48	0.304083	4.14	4.78	-0.0544547
V3	4	4.885	4.92	0.389401	4.46	5.24	-0.108418
V4	4	4.92	4.885	0.591326	4.3	5.61	0.131349
V5	4	5.91	5.88	0.175119	5.73	6.15	0.567641

There is no one transformation that will make all data sets normally distributed. Thus, the Kruskal-Wallis test must be used. Data values were converted to ranks and ANOVA was used to statistically evaluate the transformed data.

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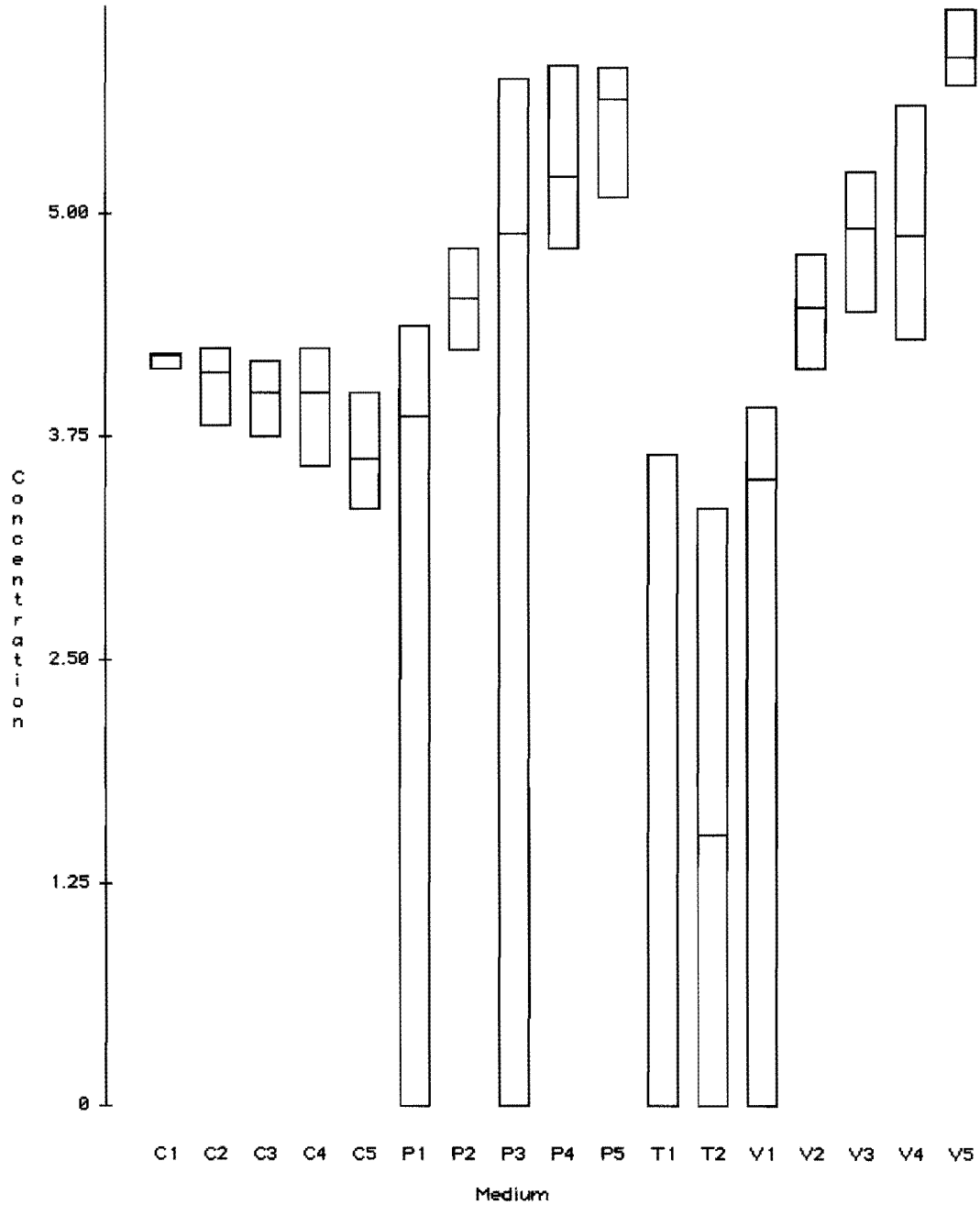
4.65
4.50
4.35
-0.75
0
0.75
nscores
P3:Concentration
5.00
3.75
2.50
1.25
0
-0.75
0
0.75
nscores
P4:Concentration
5.75
5.50
5.25
5.00
-0.75
0
0.75
nsoores
P5:Concentration
5.8
5.6
5.4
5.2
-0.75
0
0.75
nscores
T1:Concentration
3.00
2.25
1.50
0.75
0
-0.75
0
0.75
nscores
T2:Concentration
3.00
2.25
1.50
0.75
0
-0.75
0
0.75
nscores
C1:Concentration
4.22
4.20
4.18
4.16
4.14
-0.75
0
0.75
nscores
C2:Concentration
4.2
4.1
4.0
3.9
-0.75
0
0.75
nscores
C3:Concentration
4.1
4.0
3.9
3.8
-0.75
0
0.75
nscores
C4:Concentration
4.2
4.0
3.8
3.6
-0.75
0
0.75
nscores
C5:Concentration
3.90
3.75
3.60
3.45
-0.75
0
0.75
nscores



Analysis of Variance For **Rank:Concentration**
No Selector

Source	**df**	**Sums of Squares**	**Mean Square**	**F-ratio**	**Prob**
Const	1	80937	80937	850.91	≤ 0.0001
Mdm	16	21296	1331	13.993	≤ 0.0001
Error	51	4851	95.1176		
Total	67	26147			

Summary of **Rank: Concentration**
For categories in **Medium**
No Selector

Group	Count	Mean	Median	StdDev	Min	Max
C1	4	32	32.75	2.61406	28.5	34
C2	4	28.375	28.25	7.81425	19.5	37.5
C3	4	23.875	23.75	5.2341	18	30
C4	4	25.25	24.25	9.52628	15	37.5
C5	4	16.5	15.25	5.49242	11.5	24
P1	4	23.25	23.75	17.1002	4.5	41
P2	4	43.125	43.5	5.54339	36	49.5
P3	4	42.625	51.5	25.9916	4.5	63
P4	4	56.625	56	6.42099	49.5	65
P5	4	59.5	60.5	4.65475	53	64
T1	4	7.375	4.5	5.75	4.5	16
T2	4	7.375	6.75	3.47311	4.5	11.5
V1	4	14.25	14.75	8.5098	4.5	23
V2	4	40.625	43	9.0312	28.5	48
V3	4	50.25	50.5	7.5	42	58
V4	4	49.75	50	8.77021	40	59
V5	4	65.75	66.5	2.62996	62	68

$SE = \sqrt{((k(N+1))/12)}$
$SE = \sqrt{((17(69))/12)}$
$SE = 9.89$

$MSD = Q_{\alpha=0.05, k=17, df=\infty}(SE)$
$MSD = 4.792\ (9.89)$
$MSD = 47.4$

Table 8: Media and their mean ranks, with results of the Kruskal-Wallis test

Medium	Mean Rank	
T1	7.375	c
T2	7.375	c
V1	14.25	bc
C5	16.5	bc
P1	23.25	abc
C3	23.875	abc
C4	25.25	abc
C2	28.375	abc
C1	32	abc
V2	40.625	abc
P3	42.625	abc
P2	43.125	abc
V4	49.75	abc
V3	50.25	abc
P4	56.625	ab
P5	59.5	ab
V5	65.75	a

Table 9: Media and condia concentrations with results of the Kruskal-Wallis test

Medium	**Mean $\log_{10}$ conidia/mL**	
Corn Syrup (%w/v)		
0.2	4.19	abc
0.5	4.08	abc
1.0	3.98	abc
1.5	3.96	abc
2.0	3.65	bc
Pea (% v/v)		
5	3.03	abc
10	4.53	abc
15	3.89	abc
20	5.27	ab
30	5.56	ab
V8 (%v/v)		
5	2.74	bc
10	4.47	abc
15	4.89	abc
20	4.92	abc
30	5.91	a
TA (% v/v)		
33	0.91	c
100	1.60	c

Appendix C: Collego and $CaCO_3$

Table 10: Explanation of abbreviations

Medium and Concentration	Abbreviation
30% v/v filtered V8	V8
30% v/v filtered V8 with Collego additives	V8-collego
30% v/v filtered V8 with 3g/L $CaCO_3$	V8-Ca
15% v/v Pea	P
15% v/v Pea with Collego additives	P-collego
15% v/v Pea with 3g/L $CaCO_3$	P-Ca
Collego as originally formulated	COLLEGO

Table 11: Conductivity and pH of V8 and Pea media with and without Collego additives or $CaCO_3$

Medium	Conductivity (mS) pre-autoclave	Conductivity (mS) post-autoclave	pH pre-autoclave	pH post-autoclave
V8	5.33	5.57	4.13	4.28
V8-collego	18.38	19.24	6.58	6.41
V8-Ca	5.38	5.66	6.25	5.96
P	0.699	0.731	6.85	6.65
P-collego	14.70	15.19	7.49	7.03
P-Ca	0.730	0.763	7.47	7.41
COLLEGO	16.69	17.12	7.01	6.52

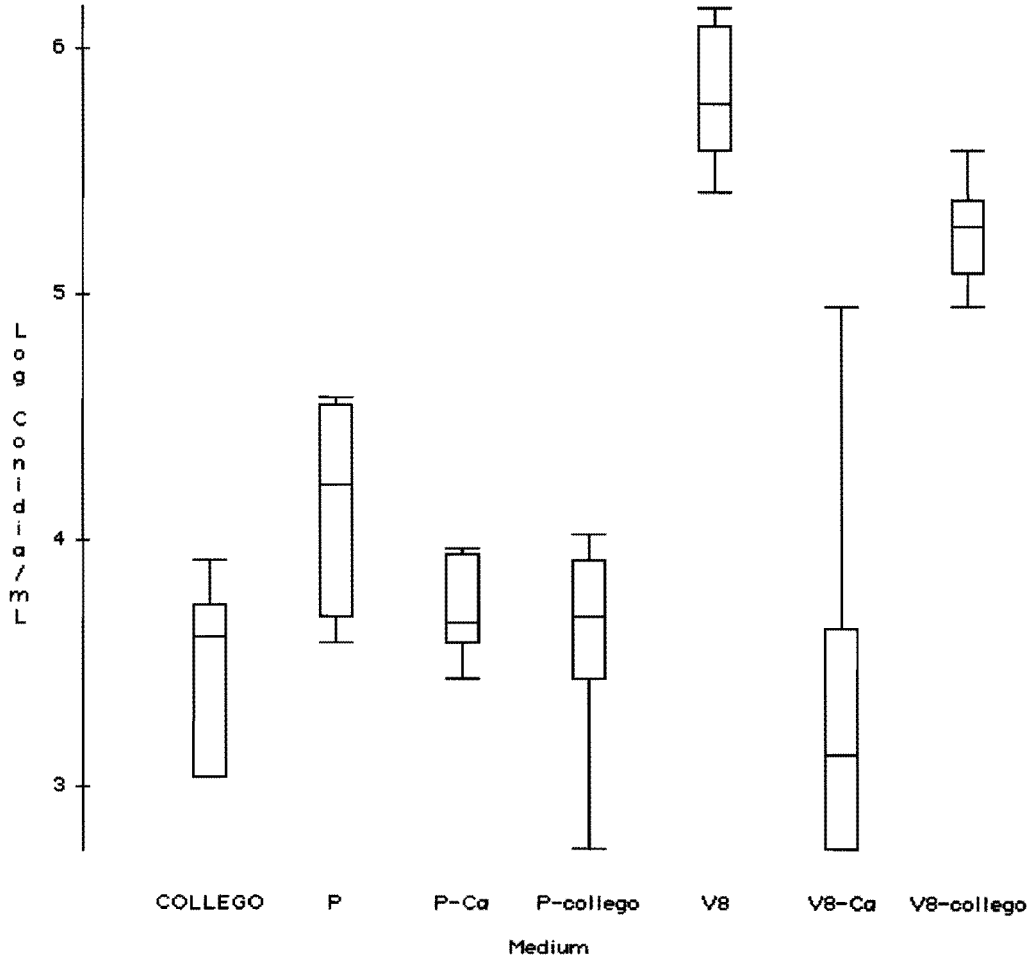
Table 12: Raw data of conidia counts in V8 and Pea with and without Collego additives or $CaCO_3$

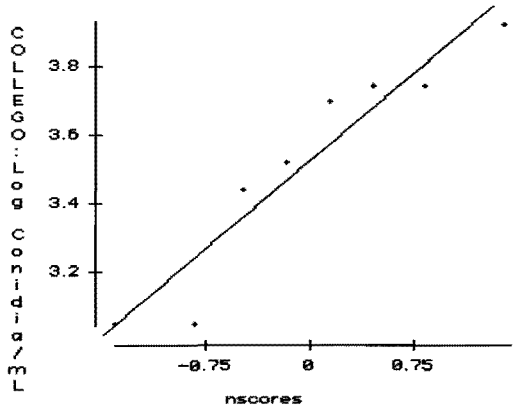
Flask Number	Flask Contents	Log_{10} conidia/mL
1	V8	5.812913
2	V8	6.068186
3	V8	5.740363
4	V8	6.089905
5	V8	5.716003
6	V8	5.414973
7	V8	5.585461
8	V8	6.164353
9	V8-collego	5.278754
10	V8-collego	5.290035
11	V8-collego	5.389166
12	V8-collego	5.243038
13	V8-collego	5.09691

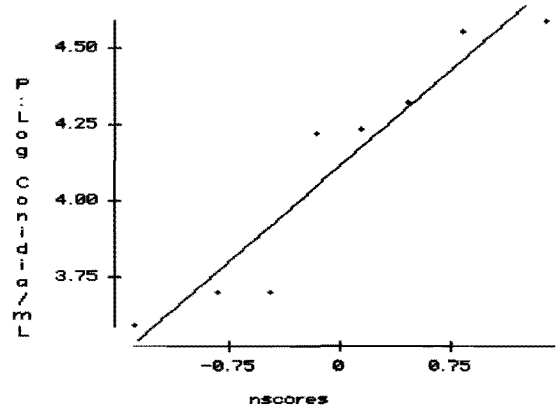
Flask Number	Flask Contents	Log_{10} conidia/mL
14	V8-collego	5.361728
15	V8-collego	5.585461
16	V8-collego	4.954243
17	V8-Ca	2.745075
18	V8-Ca	2.745075
19	V8-Ca	3.222716
20	V8-Ca	3.045323
21	V8-Ca	3.647383
22	V8-Ca	4.954243
23	V8-Ca	3.647383
24	V8-Ca	3.045323
25	COLLEGO	3.522444
26	COLLEGO	3.045323
27	COLLEGO	3.745075
28	COLLEGO	3.045323
29	COLLEGO	3.444045
30	COLLEGO	3.69897
31	COLLEGO	3.920645
32	COLLEGO	3.745075
33	P	4.58995
34	P	3.69897
35	P	4.235528
36	P	4.222716
37	P	3.69897
38	P	4.557507
39	P	3.58995
40	P	4.324282
41	P-collego	3.920645
42	P-collego	3.444045
43	P-collego	3.69897
44	P-collego	3.69897
45	P-collego	3.522444
46	P-collego	2.745075
47	P-collego	3.920645
48	P-collego	4.025306
49	P-Ca	3.58995
50	P-Ca	3.647383
51	P-Ca	3.786041
52	P-Ca	3.948902
53	P-Ca	3.647383
54	P-Ca	3.974972
55	P-Ca	3.444045
56	P-Ca	3.69897

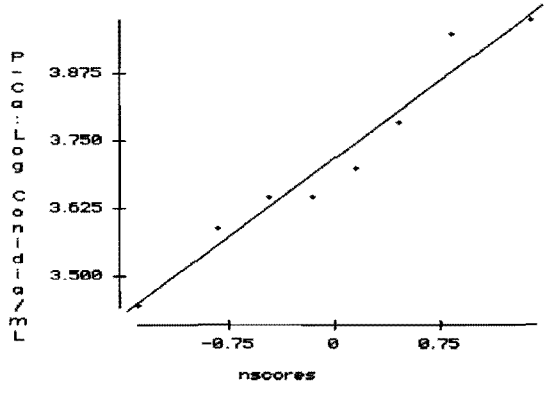
Summary of **Log Conidia/mL**
For categories in **Medium**
No Selector

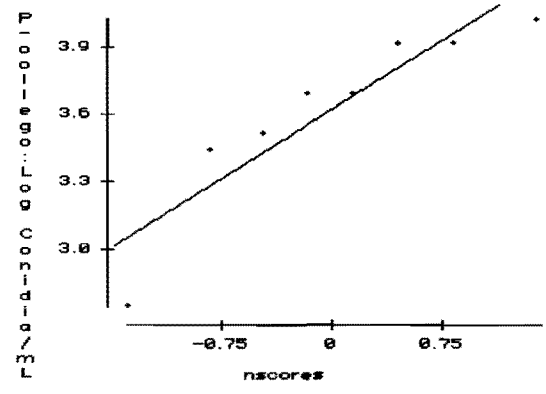
Group	Count	Mean	Median	StdDev	Min	Max
COLLEGO	8	3.52086	3.61071	0.327386	3.04532	3.92065
P	8	4.11473	4.22912	0.398776	3.58995	4.58995
P-Ca	8	3.71721	3.67318	0.179586	3.44404	3.97497
P-collego	8	3.62201	3.69897	0.407687	2.74507	4.02531
V8	8	5.82402	5.77664	0.264348	5.41497	6.16435
V8-Ca	8	3.38157	3.13402	0.724689	2.74507	4.95424
V8-collego	8	5.27492	5.28439	0.190039	4.95424	5.58546

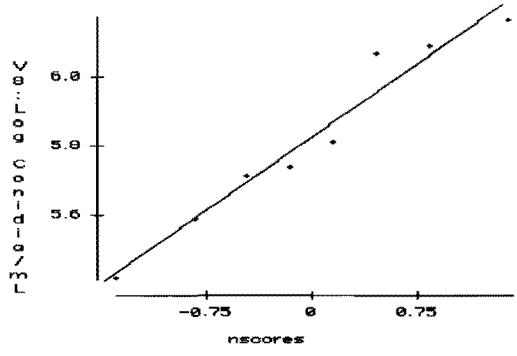


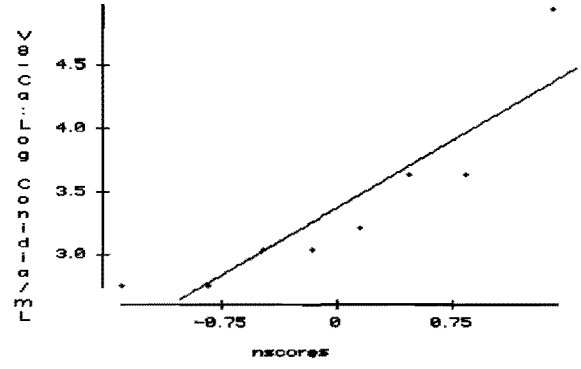

COLLEGOLog Conda/mL
3.8
3.6
3.4
3.2
-0.75
0
0.75
nscores

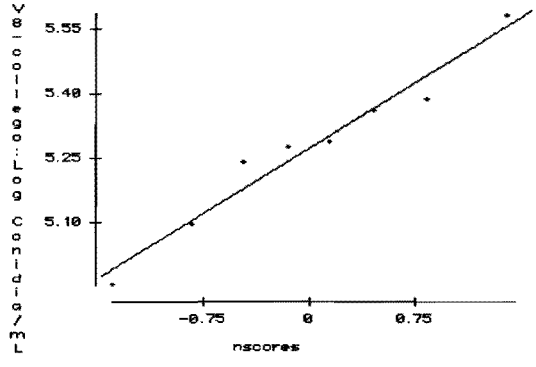

PLog Conda/mL
4.50
4.25
4.00
3.75
-0.75
0
0.75
nscores


PCaLog Conda/mL
3.875
3.750
3.625
3.500
-0.75
0
0.75
nscores


PoileoLog Conda/mL
3.9
3.6
3.3
3.0
-0.75
0
0.75
nscores


VaLog Conda/mL
6.0
5.8
5.6
-0.75
0
0.75
nscores


VaCaLog Conda/mL
4.5
4.0
3.5
3.0
-0.75
0
0.75
nscores


VaCoileoLog Conda/mL
5.55
5.40
5.25
5.10
-0.75
0
0.75
nscores

$F_{max} = S^2_{largest} / S^2_{smallest}$
$F_{max} = (0.72)^2 / (0.18)^2$
$F_{max} = 16$
$F_{critical}$ with k=7, df=7 = 11.8

There is evidence of skew in the data sets and the variances are not all equal. However, since sample sizes are the same, ANOVA can still be used with these rather modest deviations from normality and equal variance.

Analysis of Variance For **Log Conidia/mL**
No Selector

Source	df	Sums of Squares	Mean Square	F-ratio	Prob
Const	1	991.561	991.561	6333.9	≤ 0.0001
Mdm	6	43.9835	7.33059	46.827	≤ 0.0001
Error	49	7.67083	0.156548		
Total	55	51.6544			

$HSD = Q_{\alpha=0.05, k=7, df=49} \sqrt{(MSE/n_i)}$
$HSD = 4.389 \sqrt{(0.156548/8)}$
$HSD = 0.614$

Table 13: Media and condia concentrations with results of the ANOVA test

Medium	**Mean $\log_{10}$ conidia/mL**	
30% v/v filtered V8 with 3g/L $CaCO_3$	3.38	c
COLLEGO	3.52	bc
15% v/v Pea with Collego additives	3.62	bc
15% v/v Pea with 3g/L $CaCO_3$	3.72	bc
15% v/v Pea	4.11	b
30% v/v filtered V8 with Collego additives	5.27	a
30% v/v filtered V8	5.82	a

Appendix D: Filtered versus Unfiltered V8

Table 14: Explanation of Abbreviations

Medium and Concentration	Abbreviation
15% v/v filtered V8 with Collego additives	Col 15F
15% v/v unfiltered V8 with Collego additives	Col 15U
30% v/v filtered V8 with Collego additives	Col 30F
30% v/v unfiltered V8 with Collego additives	Col 30U
15% v/v filtered V8	V 15F
15% v/v unfiltered V8	V 15U
30% v/v filtered V8	V 30F
30% v/v unfiltered V8	V 30U

Table 15: Conductivity and pH of selected concentrations of filtered and unfiltered V8 with and without Collego additives

Medium	Conductivity (mS) pre-autoclave	Conductivity (mS) post-autoclave	pH pre-autoclave	pH post-autoclave
Col 15F	11.95	16.53	6.98	6.39
Col 15U	11.85	16.50	7.01	6.56
Col 30F	13.19	18.63	6.51	6.28
Col 30U	13.05	18.38	6.55	6.33
V 15F	1.93	2.76	4.27	3.96
V 15U	1.96	2.73	4.20	3.48
V 30F	3.97	5.67	4.39	3.95
V 30U	3.89	5.56	4.23	3.93

Table 16: Raw data of conidia counts at selected times post-innoculation

	Hours post-innoculation			
Medium	**43**	**78**	**102**	**136**
Col 30F	4.346352974	4.801404	5.217484	5.45
Col 30U	4.46834733	6.318063	6.201397	6.03
Col 15F	4.434568904	4.620136	4.40824	4.24
Col 15U	4.84509804	5.607455	5.667453	5.71
V 30F	4.086359831	4.206826	4.85187	4.86
V 30U	4.460897843	6.763428	6.829304	6.67
V 15F	4.357934847	4.83123	5.875061	5.92
V 15U	4.086359831	6.403121	6.537819	6.53

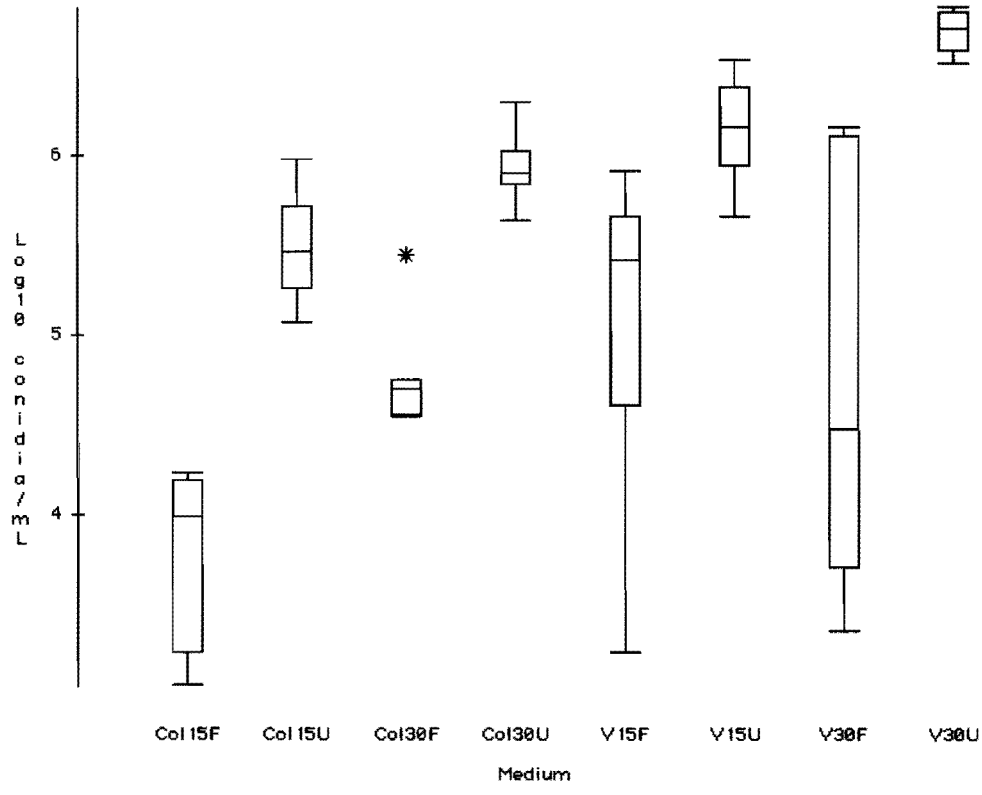
Table 17: Raw data of conidia counts in selected concentrations of filtered and unfilteredV8 with and without Collego additives

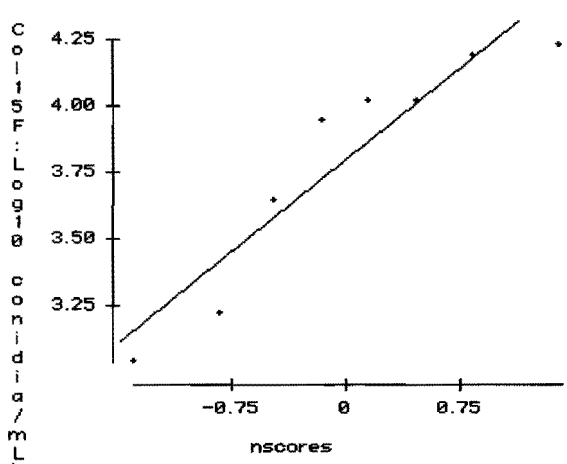
Flask Number	Flask Contents	Log_{10} conidia/mL
1	Col30F	4.72
2	Col30F	4.55
3	Col30F	4.64
4	Col30F	4.73
5	Col30F	4.68
6	Col30F	4.75
7	Col30F	4.56
8	Col30F	5.45
9	Col30U	5.90
10	Col30U	5.85
11	Col30U	5.90
12	Col30U	5.87
13	Col30U	6.30
14	Col30U	6.00
15	Col30U	5.64
16	Col30U	6.03
17	Col15F	3.05
18	Col15F	3.65
19	Col15F	4.19
20	Col15F	3.22
21	Col15F	4.03
22	Col15F	3.95
23	Col15F	4.03
24	Col15F	4.24
25	Col15U	5.27
26	Col15U	5.35
27	Col15U	5.42
28	Col15U	5.98
29	Col15U	5.72
30	Col15U	5.08
31	Col15U	5.51
32	Col15U	5.71
33	V30F	3.35
34	V30F	6.15
35	V30F	4.60
36	V30F	3.70
37	V30F	4.18
38	V30F	4.37
39	V30F	6.10
40	V30F	4.86

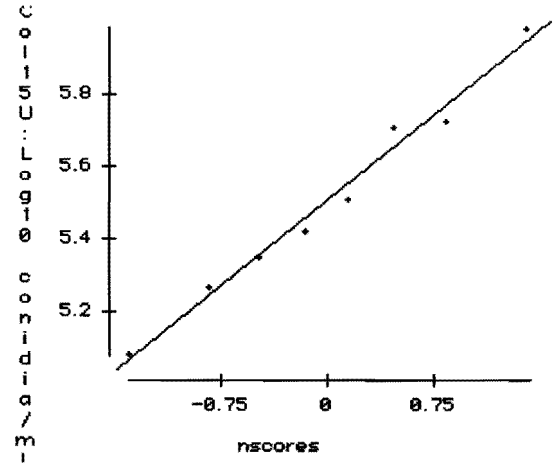
Flask Number	Flask Contents	Log_{10} conidia/mL
41	V30U	6.51
42	V30U	6.58
43	V30U	6.72
44	V30U	6.82
45	V30U	6.72
46	V30U	6.69
47	V30U	6.80
48	V30U	6.67
49	V15F	5.66
50	V15F	5.37
51	V15F	5.63
52	V15F	5.47
53	V15F	4.61
54	V15F	5.34
55	V15F	3.22
56	V15F	5.92
57	V15U	6.37
58	V15U	6.37
59	V15U	5.95
60	V15U	6.22
61	V15U	6.09
62	V15U	5.66
63	V15U	6.06
64	V15U	6.53

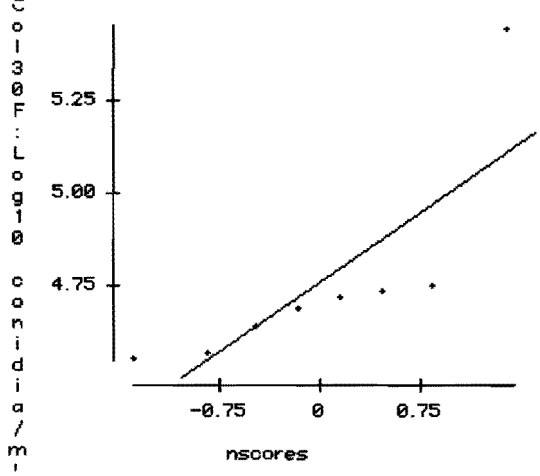
Summary of **Log10 conidia/mL**
For categories in **Medium**
No Selector

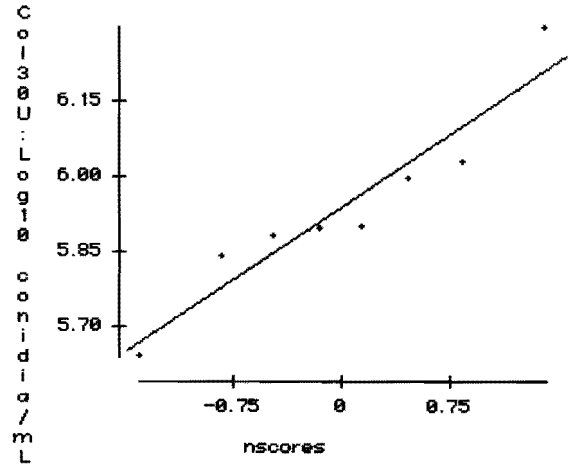
Group	Count	Mean	Median	StdDev	Min	Max
Col15F	8	3.79295	3.9871	0.446193	3.04532	4.23553
Col15U	8	5.50678	5.46756	0.29001	5.07918	5.98453
Col30F	8	4.76067	4.70081	0.287358	4.55023	5.44716
Col30U	8	5.93745	5.90173	0.186876	5.63849	6.29667
V15F	8	5.15281	5.42044	0.868085	3.22272	5.91645
V15U	8	6.15714	6.15192	0.277716	5.66276	6.53148
V30F	8	4.66198	4.48143	1.0228	3.34635	6.15229
V30U	8	6.68841	6.70429	0.103273	6.5092	6.81823

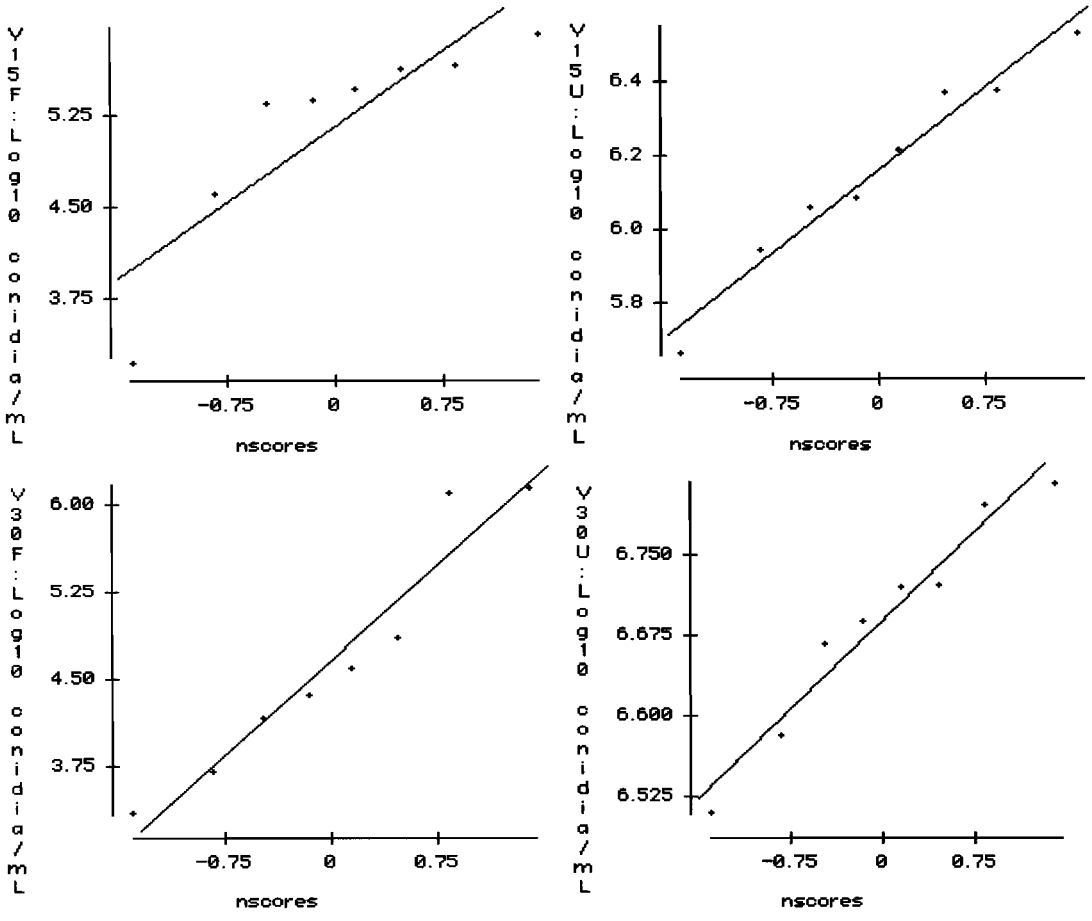

Log10 conidia/mL
6
5
4
Col15F
Col15U
Col30F
Col30U
V15F
V15U
V30F
V30U
Medium


Col15F:Log10 conidia/mL
4.25
4.00
3.75
3.50
3.25
-0.75
0
0.75
nscores


Col15U:Log10 conidia/mL
5.8
5.6
5.4
5.2
-0.75
0
0.75
nscores


Col30F:Log10 conidia/mL
5.25
5.00
4.75
-0.75
0
0.75
nscores


Col30U:Log10 conidia/mL
6.15
6.00
5.85
5.70
-0.75
0
0.75
nscores



The 30% V8 with collego additives treatment has a large positive outlier that is not eliminated by data transformation. With such an extreme violation of normality, the Kruskal-Wallis test must be used. Data values were converted to ranks and ANOVA was used to statistically evaluate the transformed data.

Analysis of Variance For **Rank:Log10 conidia/mL**
No Selector

Source	**df**	**Sums of Squares**	**Mean Square**	**F-ratio**	**Prob**
Const	1	67600	67600	787.55	≤ 0.0001
Mdm	7	17032.2	2433.17	28.347	≤ 0.0001
Error	56	4806.81	85.8359		
Total	63	21839			

Summary of **Rank:Log10 conidia/mL**
For categories in **Medium**
No Selector

Group	**Count**	**Mean**	**Median**	**StdDev**	**Min**	**Max**
Col15F	8	6.9375	7.75	3.88622	1	12
Col15U	8	32.125	30.5	7.33753	24	45
Col30F	8	19.875	19.5	4.94072	14	30
Col30U	8	42.75	41.5	5.80025	34	53
V15F	8	26.9375	29.5	12.3879	2.5	43
V15U	8	49.375	50.5	6.84392	36	57
V30F	8	21.625	14.5	18.7688	4	51
V30U	8	60.375	60.5	2.66927	56	64

$SE = \sqrt{((k(N+1))/12)}$
$SE = \sqrt{((8(65))/12)}$
$SE = 6.58$

$MSD = Q_{\alpha=0.05, k=8, df=\infty}(SE)$
$MSD = 4.286\ (6.58)$
$MSD = 28.21$

Table 18: Media and their mean ranks, with results of the Kruskal-Wallis test

Treatment	Mean Rank	
Col 15F	6.94	d
Col 30F	19.88	cd
V 30F	21.63	bcd
V 15F	26.94	bcd
Col 15U	32.13	bcd
Col 30U	42.75	abc
V 15U	49.38	ab
V 30U	60.38	a

Table 20: Mean conidia concentration for filtered and unfiltered V8, with or without Collego additives, and results of the Kruskal-Wallis test

Medium and Concentration	Mean Log_{10} conidia/mL	
15% v/v filtered V8 with Collego additives	3.79	d
15% v/v unfiltered V8 with Collego additives	5.51	bcd
30% v/v filtered V8 with Collego additives	4.76	cd
30% v/v unfiltered V8 with Collego additives	5.94	abc
15% v/v filtered V8	5.15	bcd
15% v/v unfiltered V8	6.16	ab
30% v/v filtered V8	4.66	bcd
30% v/v unfiltered V8	6.69	a

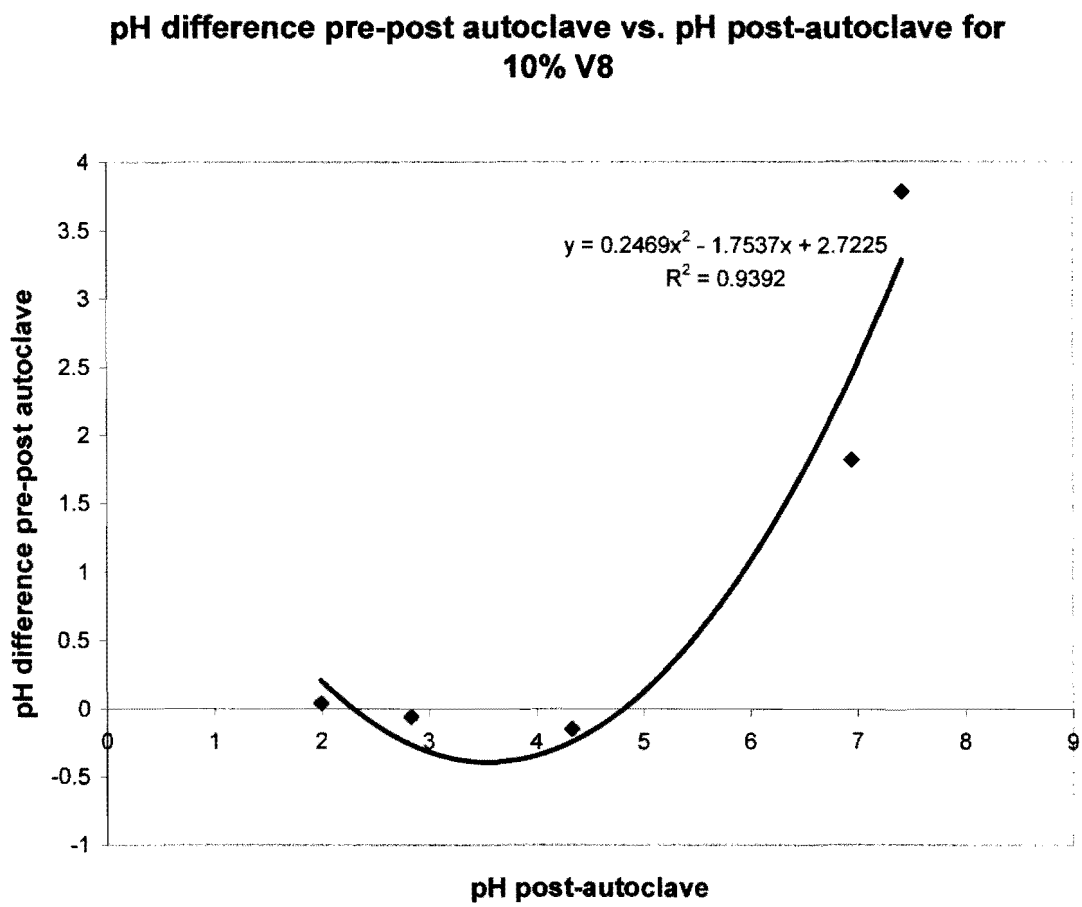
Appendix E: pH and Conductivity Calibrations

Table 21: Results of pH and conductivity calibrations for selected concentrations of V8

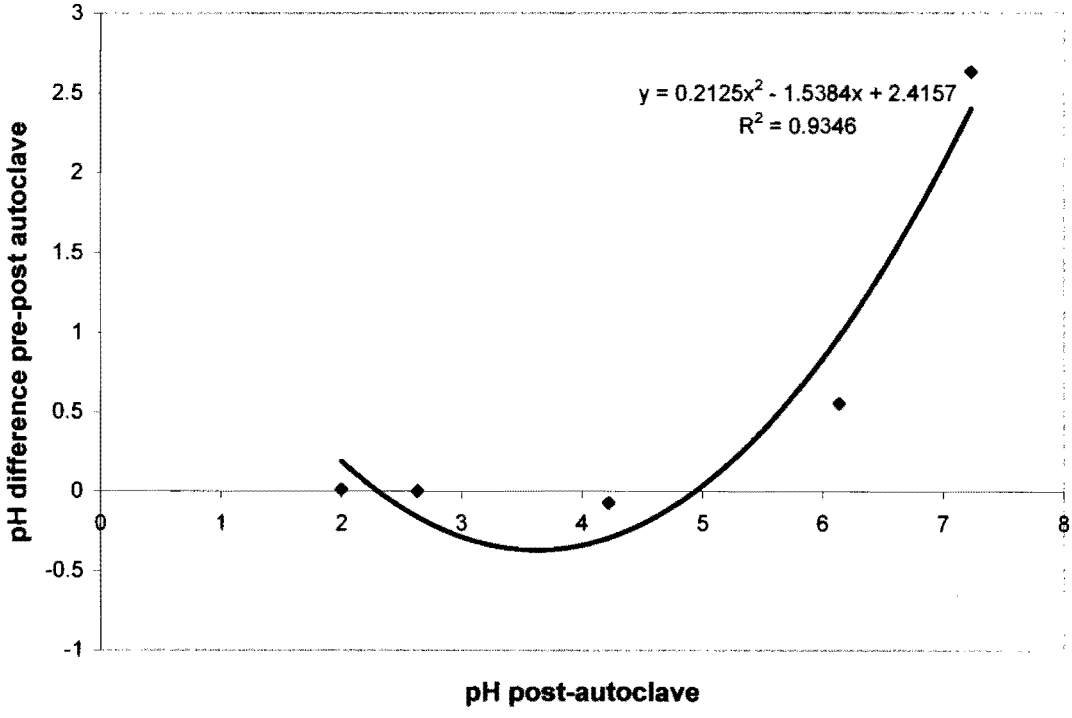
Flask Number	% V8	pH pre-autoclave	pH post-autoclave	Grams KCl added	Conductivity pre-autoclave(mS)	Conductivity post-autoclave(mS)
1	10	4.18	4.33	--	--	--
2	10	2.77	2.83	--	--	--
3	10	2.03	1.99	--	--	--
4	10	8.76	6.94	--	--	--
5	10	11.19	7.41	--	--	--
6	10	--	--	0	1.87	1.96
7	10	--	--	0.5	9.20	10.29
8	10	--	--	1	15.99	17.86
9	10	--	--	1.5	22.7	25.6
10	10	--	--	2	29.3	32.6
11	15	4.15	4.22	--	--	--
12	15	2.63	2.63	--	--	--
13	15	2.01	2.00	--	--	--
14	15	6.69	6.14	--	--	--
15	15	9.86	7.23	--	--	--
16	15	--	--	0	2.54	2.87
17	15	--	--	0.5	10.36	11.52
18	15	--	--	1	15.98	18.00
19	15	--	--	1.5	23.4	26.2
20	15	--	--	2	29.4	32.8
21	30	4.11	4.09	--	--	--
22	30	3.08	3.09	--	--	--
23	30	2.01	2.01	--	--	--
24	30	8.86	6.65	--	--	--
25	30	9.98	7.05	--	--	--
26	30	--	--	0	4.74	5.32
27	30	--	--	0.5	11.74	12.97
28	30	--	--	1	18.35	20.5
29	30	--	--	1.5	25.1	27.9
30	30	--	--	2	31.0	34.5
31	50	4.03	4.02	--	--	--
32	50	2.26	2.30	--	--	--
33	50	3.08	3.22	--	--	--
34	50	7.78	6.29	--	--	--
35	50	9.07	6.84	--	--	--
36	50	--	--	0	7.38	8.15

Flask Number	% V8	pH pre-autoclave	pH post-autoclave	Grams KCl added	Conductivity pre-autoclave(mS)	Conductivity post-autoclave(mS)
37	50	--	--	0.5	14.17	15.80
38	50	--	--	1	21.0	22.9
39	50	--	--	1.5	26.6	29.5
40	50	--	--	2	33.0	36.1
41	75	3.94	3.91	--	--	--
42	75	2.94	2.96	--	--	--
43	75	2.27	2.22	--	--	--
44	75	6.60	5.84	--	--	--
45	75	9.42	7.18	--	--	--
46	75	--	--	0	10.30	11.18
47	75	--	--	0.5	16.57	18.1
48	75	--	--	1	22.8	25.0
49	75	--	--	1.5	28.7	32.2
50	75	--	--	2	34.5	37.5

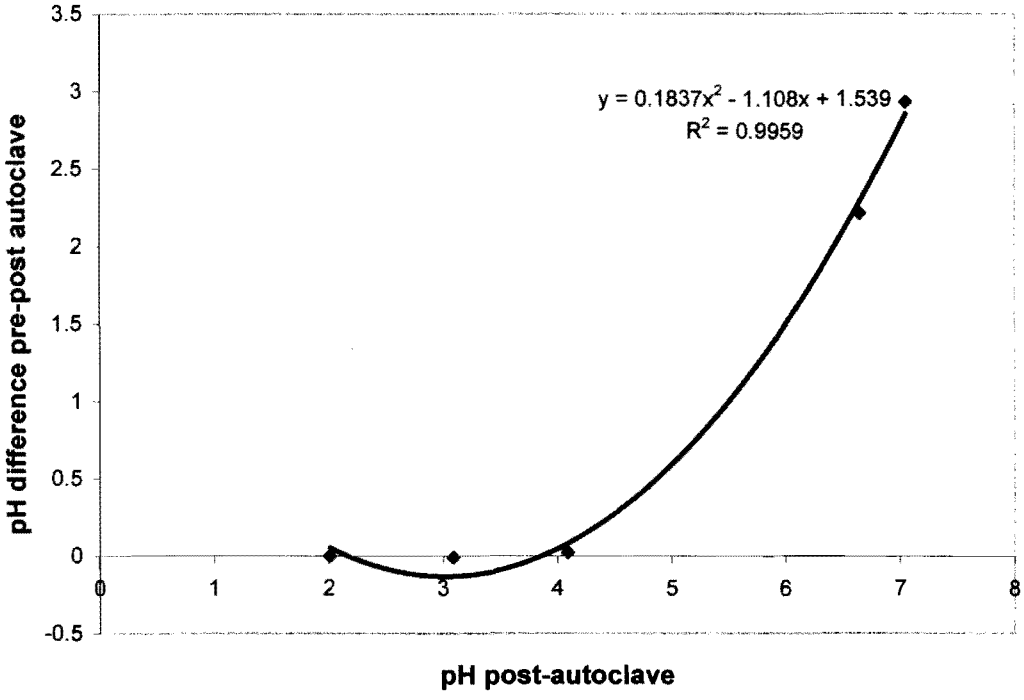
Figure 3: Plotted pre-post autoclave differences vs. post-autoclave readings



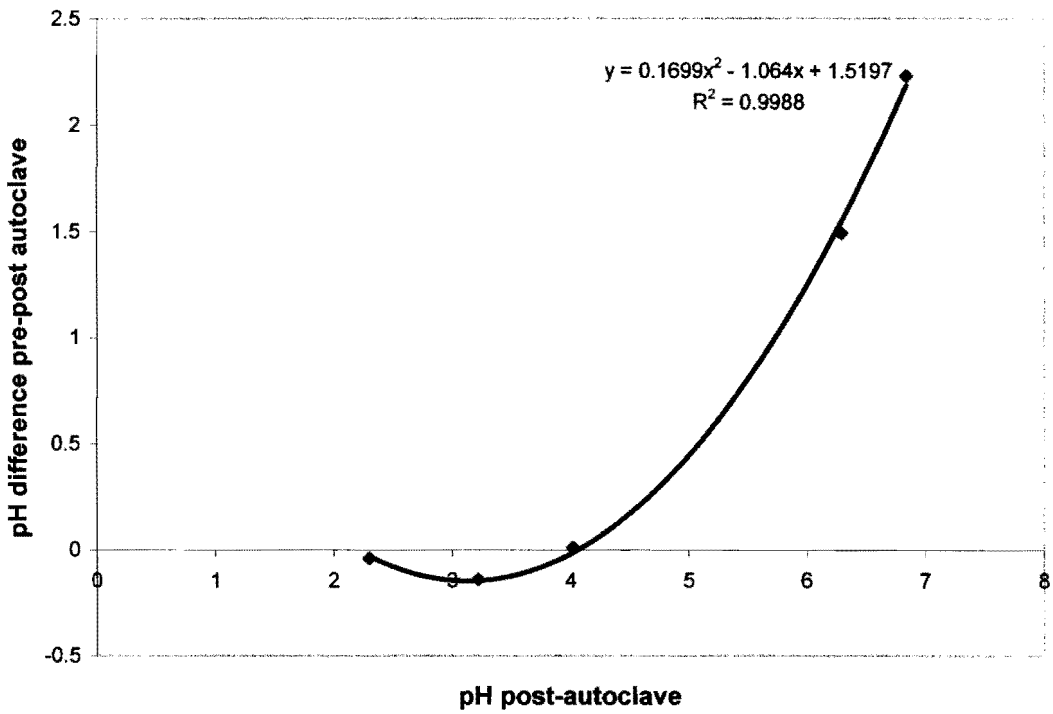
pH difference pre-post autoclave vs. pH post-autoclave for 15% V8



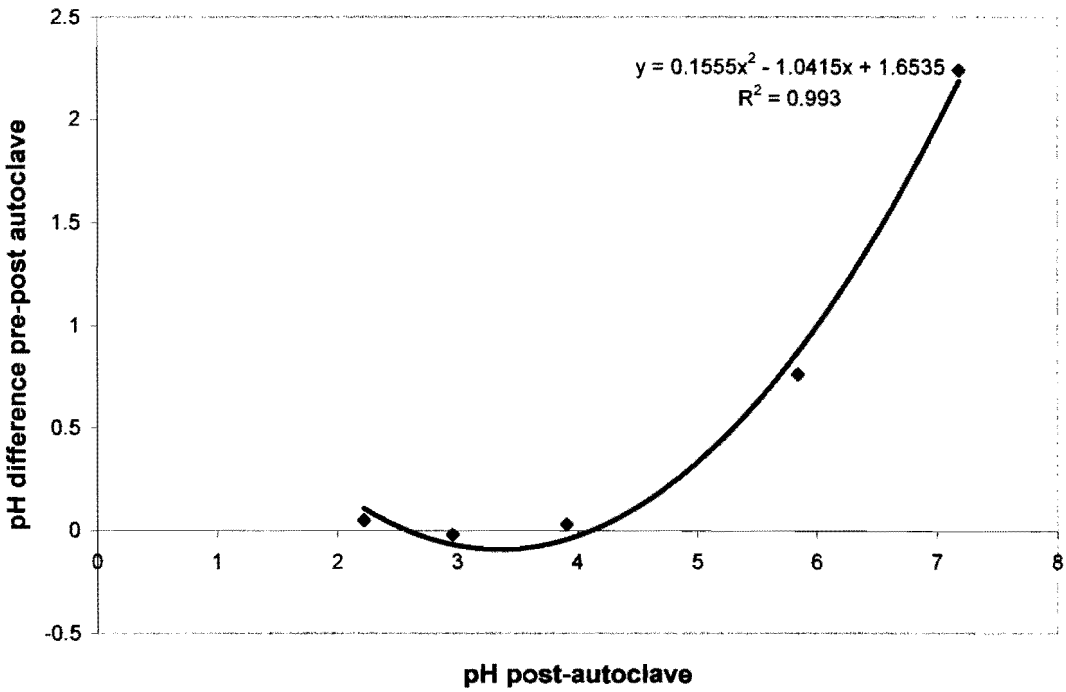
pH difference pre-post autoclave vs. pH post-autoclave for 30% V8



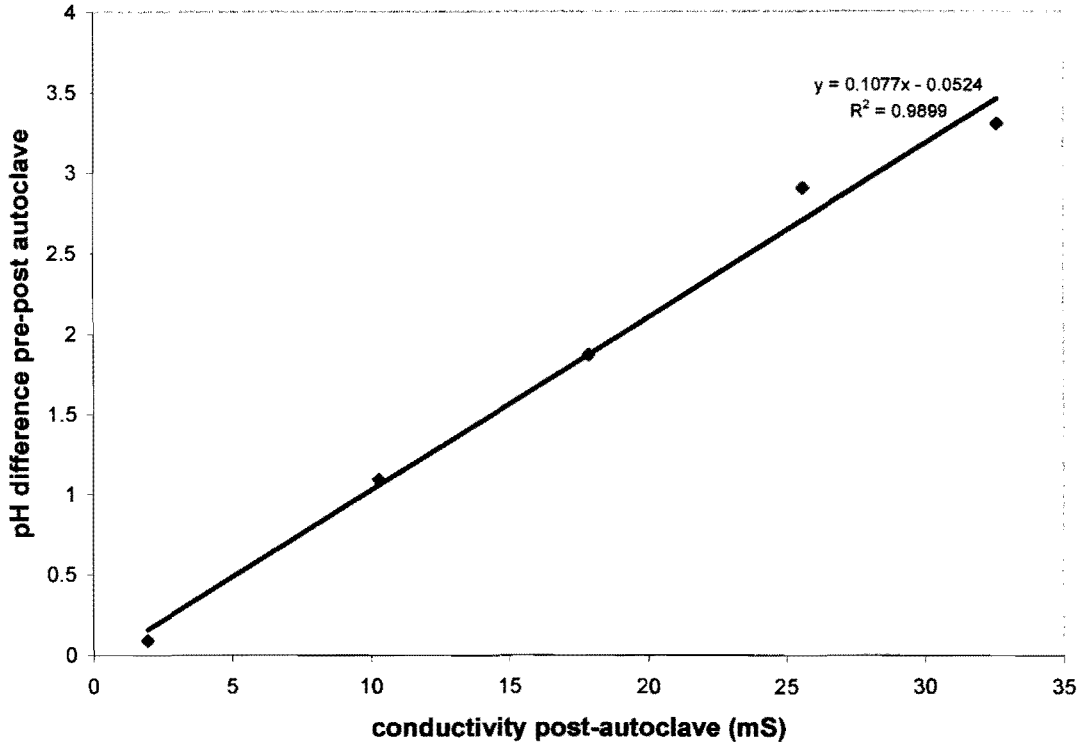
pH difference pre-post autoclave vs. pH post-autoclave for 50% V8



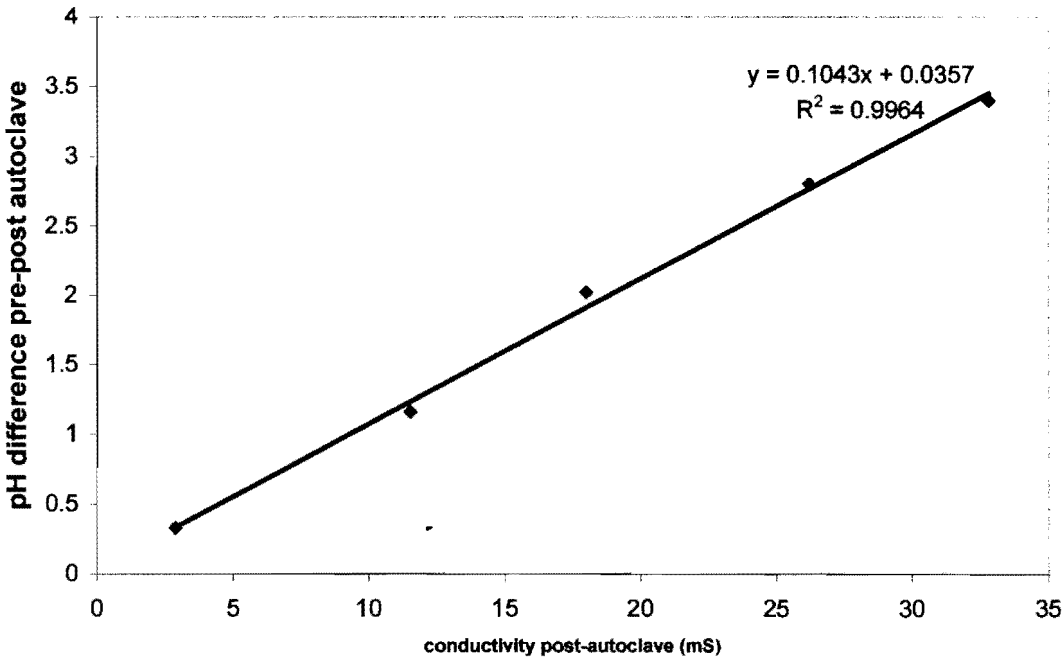
pH difference pre-post autoclave vs. pH post-autoclave for 75% V8



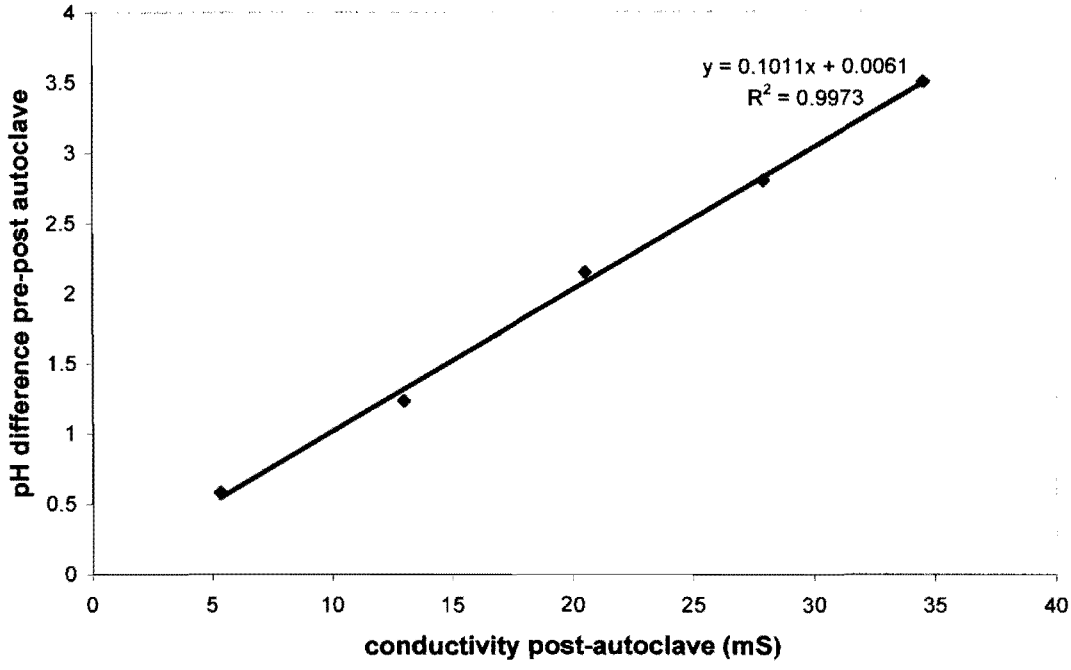
conductivity difference pre-post autoclave vs. conductivity post-autoclave for 10% V8



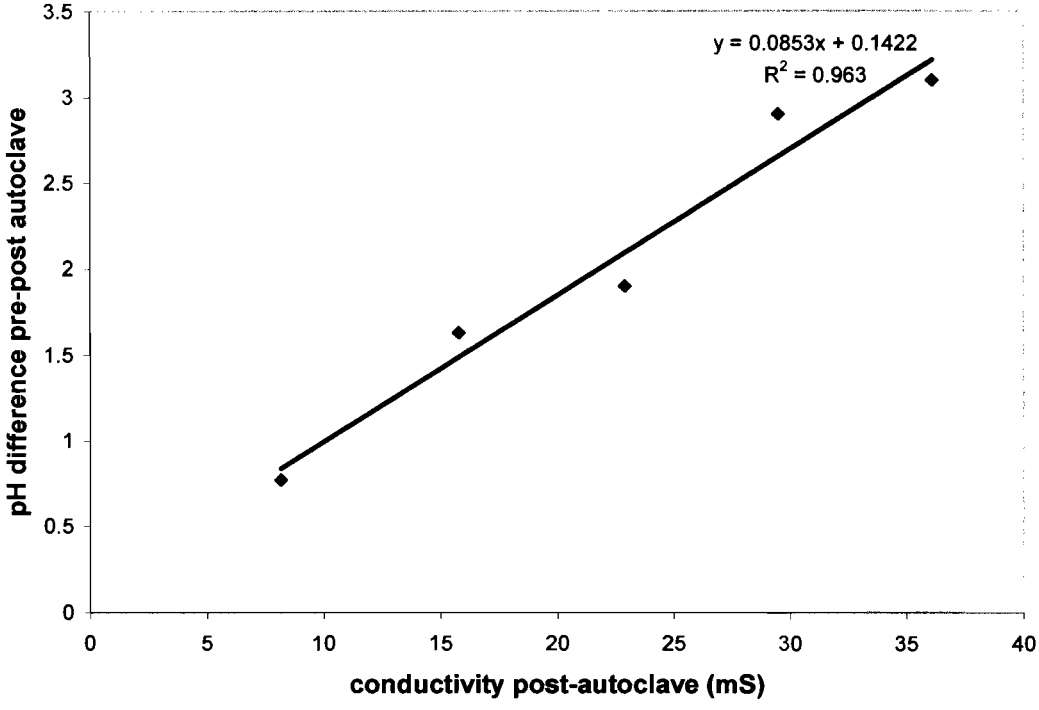
conductivity difference pre-post autoclave vs. conductivity post-autoclave for 15% V8



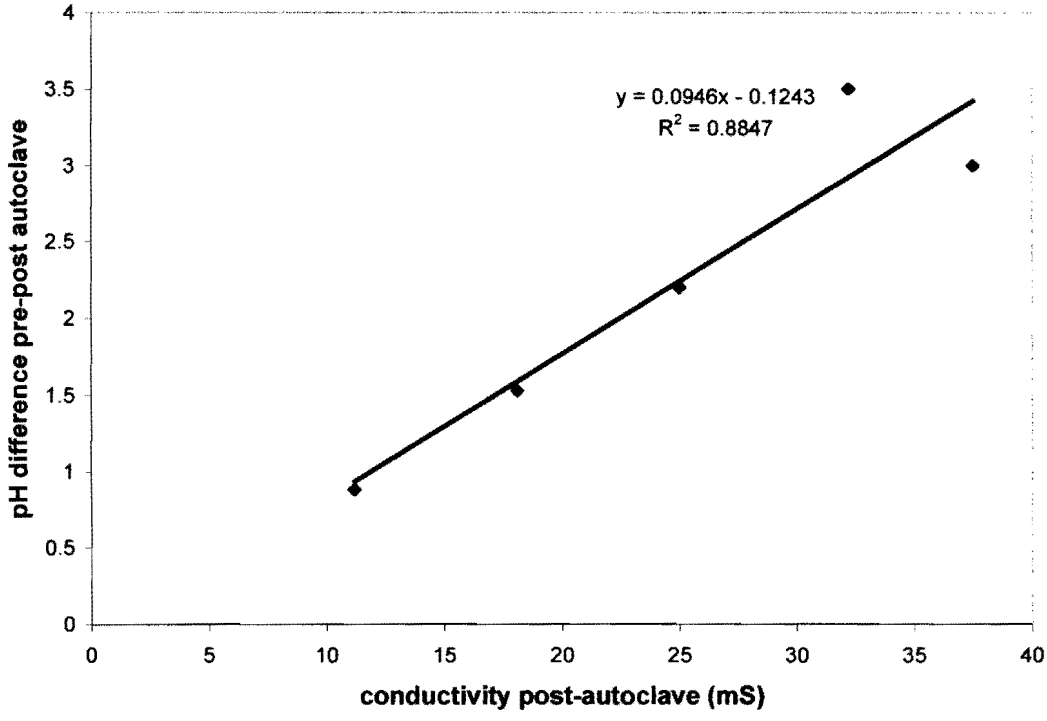
conductivity difference pre-post autoclave vs. conductivity post-autoclave for 30% V8



conductivity difference pre-post autoclave vs. conductivity post-autoclave for 50% V8



conductivity difference pre-post autoclave vs. conductivity post-autoclave for 75% V8



Appendix F: V8 Concentration, pH, and Conductivity

Table 22: Adjustments in pH to achieve desired post-autoclave pH

V8 concentration	Desired pH	Adjusted pH pre-autoclave
18%	3	2.713
18%	6.5	7.9
53%	3	2.86
53%	6.5	8.28
13%	4.75	4.65
58%	4.75	5.95
35.5%	2.5	2.42
35.5%	7	9.78
35.5%	4.75	5.17

Table 23: Adjustments in conductivity to achieve desired post-autoclave conductivity

V8 concentration	Desired Conductivity (mS)	Adjusted Conductivity pre-autoclave (mS)
18%	12	10.71
18%	38	34
53%	12	10.83
53%	38	34.62
13%	25	22.36
58%	25	22.73
35.5%	25	22.47
35.5%	8.3	7.45
35.5%	41.7	37.48

Table 24: Target pH and conductivity, actual pH and conductivity, conidia concentration, and media color

		Target		Actual			
Flask Number	V8 %	pH	Conductivity	pH	Conductivity	Log_{10} conidia/mL	Medium color
1	18%	3	12	3.4	11.40	3.70	Tan
2	18%	3	38	3.5	36.1	3.70	L orange
3	18%	6.5	12	6.6	11.40	6.24	D green
4	18%	6.5	38	6.8	36.0	6.71	Brown
5	53%	3	12	3.4	11.52	4.00	D green
6	53%	3	38	3.6	36.5	4.07	Orange
7	53%	6.5	12	6.8	11.35	6.59	D green
8	53%	6.5	38	7.0	36.7	7.15	Brown
9	13%	4.75	25	4.9	24.1	6.53	Olive gr.
10	58%	4.75	25	6.0	23.9	7.20	Brown

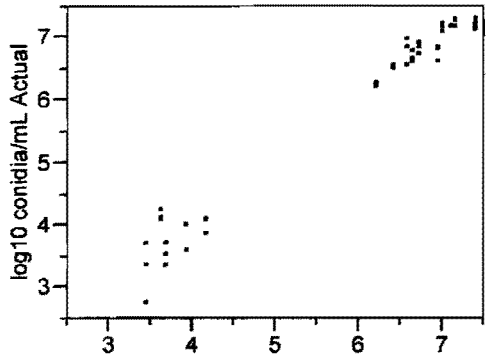
		Target		Actual			
Flask Number	**V8 %**	**pH**	**Conductivity**	**pH**	**Conductivity**	**Flask Number**	**V8 %**
11	35.5%	2.5	25	3.1	24.0	4.12	Orange
12	35.5%	7.0	25	7.4	23.7	6.79	Brown
13	35.5%	4.75	8.27	5.1	7.87	6.95	D green
14	35.5%	4.75	41.73	5.6	39.5	7.15	Brown
15	35.5%	4.75	25	5.5	23.7	7.13	Brown
16	35.5%	4.75	25	5.5	23.7	7.19	Brown
17	18%	3	12	3.4	11.40	2.74	Tan
18	18%	3	38	3.5	36.1	3.52	L orange
19	18%	6.5	12	6.6	11.40	6.20	Olive gr.
20	18%	6.5	38	6.8	36.0	6.90	Brown
21	53%	3	12	3.4	11.52	3.59	D green
22	53%	3	38	3.6	36.5	4.09	Orange
23	53%	6.5	12	6.8	11.35	6.76	D green
24	53%	6.5	38	7.0	36.7	7.27	Brown
25	13%	4.75	25	4.9	24.1	6.54	Olive gr.
26	58%	4.75	25	6.0	23.9	7.07	brown
27	35.5%	2.5	25	3.1	24.0	4.24	Orange
28	35.5%	7.0	25	7.4	23.7	6.81	Brown
29	35.5%	4.75	8.27	5.1	7.87	6.54	D green
30	35.5%	4.75	41.73	5.6	39.5	7.16	Brown
31	35.5%	4.75	25	5.5	23.7	7.28	Brown
32	35.5%	4.75	25	5.5	23.7	7.17	Brown
33	18%	3	12	3.4	11.40	3.35	Tan
34	18%	3	38	3.5	36.1	3.35	L orange
35	18%	6.5	12	6.6	11.40	6.26	Olive gr.
36	18%	6.5	38	6.8	36.0	6.83	Brown
37	53%	3	12	3.4	11.52	3.59	D green
38	53%	3	38	3.6	36.5	3.86	Orange
39	53%	6.5	12	6.8	11.35	6.64	D green
40	53%	6.5	38	7.0	36.7	7.24	Brown
41	13%	4.75	25	4.9	24.1	6.49	Olive gr.
42	58%	4.75	25	6.0	23.9	7.15	Brown
43	35.5%	2.5	25	3.1	24.0	4.09	Orange
44	35.5%	7.0	25	7.4	23.7	6.60	Olive gr.
45	35.5%	4.75	8.27	5.1	7.87	6.82	D green
46	35.5%	4.75	41.73	5.6	39.5	7.16	Brown
47	35.5%	4.75	25	5.5	23.7	7.10	Brown
48	35.5%	4.75	25	5.5	23.7	7.22	Brown

Figure 4: Orthagonal CCD analysis of data in table 24

Response log10 conidia/mL

Whole Model

Actual by Predicted Plot



log10 conidia/mL Predicted P<.0001
RSq=0.97 RMSE=0.268

Summary of Fit

RSquare	0.974558
RSquare Adj	0.968532
Root Mean Square Error	0.268049
Mean of Response	5.892708
Observations (or Sum Wgts)	48

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	104.58464	11.6205	161.7324
Error	38	2.73031	0.0719	Prob > F
C. Total	47	107.31495		<.0001

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	5	1.8510273	0.370205	13.8940
Pure Error	33	0.8792833	0.026645	Prob > F
Total Error	38	2.7303107		<.0001
				Max RSq
				0.9918

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	6.7904008	0.090169	75.31	<.0001
V8(18,53)&RS	0.2318244	0.048815	4.75	<.0001
pH(3,6.5)&RS	2.1105324	0.058234	36.24	<.0001
conductivity (mS)(12,38)&RS	0.112163	0.052671	2.13	0.0398
V8(18,53)*pH(3,6.5)	-0.013438	0.057443	-0.23	0.8163
V8(18,53)*conductivity (mS)(12,38)	-0.000415	0.057178	-0.01	0.9942
pH(3,6.5)*conductivity (mS)(12,38)	0.0774555	0.060033	1.29	0.2048
V8(18,53)*V8(18,53)	-0.416562	0.066175	-6.29	<.0001
pH(3,6.5)*pH(3,6.5)	-1.320171	0.073988	-17.84	<.0001
conductivity (mS)(12,38)*conductivity (mS)(12,38)	-0.373732	0.073176	-5.11	<.0001

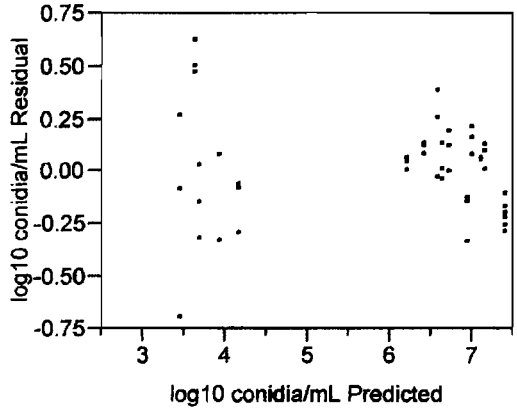
Response log10 conidia/mL

Whole Model

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
V8(18,53)&RS	1	1	1.620470	22.5534	<.0001
pH(3,6.5)&RS	1	1	94.376469	1313.516	<.0001
conductivity (mS)(12,38)&RS	1	1	0.325819	4.5347	0.0398
V8(18,53)*pH(3,6.5)	1	1	0.003932	0.0547	0.8163
V8(18,53)*conductivity (mS)(12,38)	1	1	0.000004	0.0001	0.9942
pH(3,6.5)*conductivity (mS)(12,38)	1	1	0.119605	1.6646	0.2048
V8(18,53)*V8(18,53)	1	1	2.847090	39.6253	<.0001
pH(3,6.5)*pH(3,6.5)	1	1	22.875255	318.3739	<.0001
conductivity (mS)(12,38)*conductivity (mS)(12,38)	1	1	1.874161	26.0843	<.0001

Residual by Predicted Plot



Response Surface

Coef

	V8(18,53)	pH(3,6.5)	conductivity (mS)(12,38)	log10 conidia/mL
V8(18,53)	-0.416562	-0.013438	-0.000415	0.2318244
pH(3,6.5)	.	-1.320171	0.0774555	2.1105324
conductivity (mS)(12,38)	.	.	-0.373732	0.112163

Solution

Variable	Critical Value
V8(18,53)	40.140324
pH(3,6.5)	6.1584622
conductivity (mS)(12,38)	28.033043

Solution is a Maximum

Predicted Value at Solution 7.6835364

Figure 5: Contour Profiler

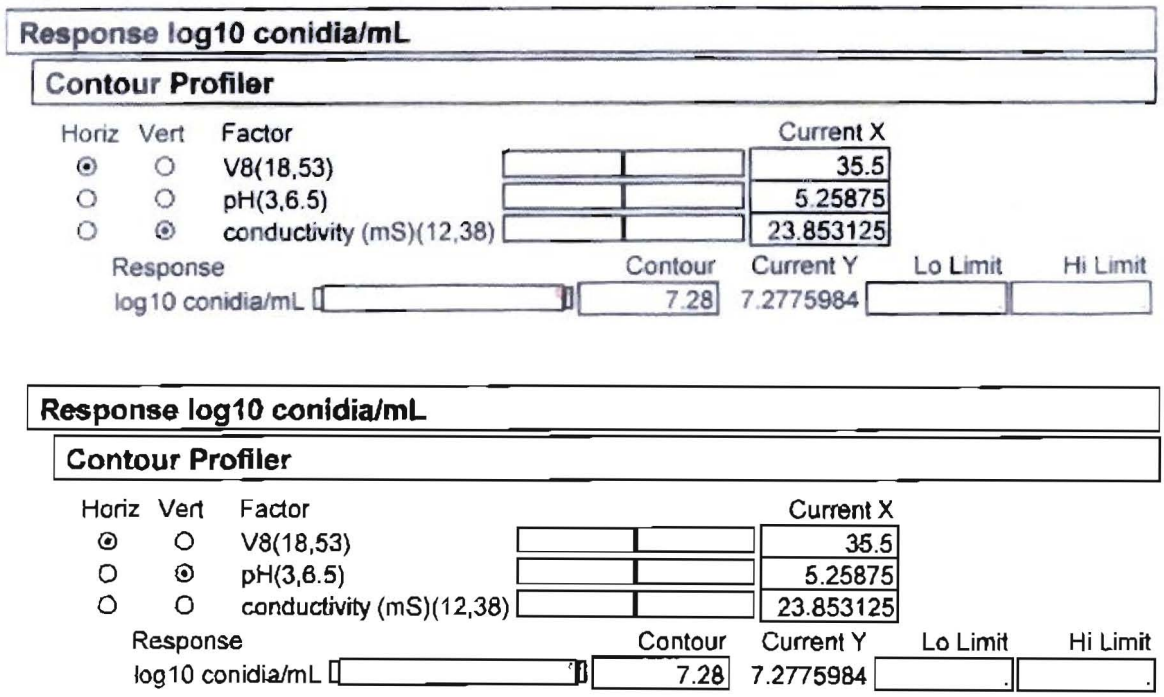


Figure 6: Prediction profiler with maximized desirability